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Report

FINAL REPORT

DEVELOPMENT OF BIOLOGICAL
AND NONBIOLOGICAL EXPLANATIONS
FOR THE VIKING LABELED
RELEASE DATA

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TABLE OF CONTENTS

BIOSPHERICS INCORPORATED

LIST OF FIGURES

BIOSPHERICS INCORPORATED

LIST OF TABLES

BIOSPHERICS INCORPORATED

I. Summary

This report covers the final year of an intensive three
year post-Viking effort to develop nonbiological explanations
of the positive responses obtained on Mars by the Labeled Release
life detection experiment. During the study, a variety of
hypotheses were explored. All were contraindicated by experimental
evidence with the exception of one which postulates hydrogen
peroxide as the active agent. During this past year, an intensive
investigation was continued to examine the plausibility that
hydrogen peroxide, widely distributed within the Mars surface
material, was responsible for the evocative responses obtained by
the Viking Labeled Release (LR) experiment on Mars.

Our results have shown that hydrogen peroxide in aqueous
solution at a concentration of 10^{-1}M can react with the LR
nutrient in a flight-like instrument to produce a positive
response similar to that obtained during the mission. The hydrogen
peroxide concentration required to produce the positive response
can be lowered by an order of magnitude or more, if a metal
catalyst is introduced to stimulate the reaction. Also, it is
dissipated by three hours heat treatment at 160°C to fulfill the
"control requirement". However, to make hydrogen peroxide in a
flight-like instrument fulfill the thermal criteria of the
Viking LR is more difficult. When heated for three hours
at 50°C, it is volatilized out of the test cell so that,
on flight, it should not have been available for subsequent
reaction with the LR nutrient. Experiments conducted by placing

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hydrogen peroxide on an appropriate soil sample partially stabilized it to volatilization at $50^{\circ}C$. Thus, if an appropriate complex exists on Mars between hydrogen peroxide and the surface material, it would be possible to obtain partial reduction of activity with the LR nutrient following the $50^{\circ}C$ heating regime.

With regards to stability, hydrogen peroxide at room temperature is stable on low pH soils but not on neutral or alkaline soils. Lowering the temperature to $-70^{\circ}C$ preserves hydrogen peroxide on neutral or high pH soils, suggesting that if a mechanism exists for formation of hydrogen peroxide on Mars, then the low ambient surface temperatures would contribute to its stability and accumulation. After low temperature storage on select soil, hydrogen peroxide can partially survive the subsequent temperature rise to $10^{\circ}C$ for the two sols prior to nutrient addition. However, we estimate that this would require concentrations of hydrogen peroxide on the surface of Mars in the order of 1.0M. Further, the destructive effect on hydrogen peroxide of the ultraviolet flux incident to the Mars surface would have to be mitigated. No solution to this problem has been found.

Two major problems stand in the way of the hydrogen peroxide theory: (1) demonstration that it is formed or deposited on the Mars surface in quantities sufficient to account for the LR flight data (no experimental evidence has yet accounted for largescale hydrogen peroxide formation) and (2) since hydrogen peroxide is highly susceptible to ultraviolet radiation, some

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yet unknown method for preserving it on the surface in
considerable excers over that required for the LR experiment
must be postulated. In order for the hydrogen peroxide theory
to be compatible with laboratory and flight data, the Mars
surface material must catalyze the reaction between hydrogen
peroxide and the LR nutrient, and must also form a complex
with hydrogen peroxide that will stabilize it to ultraviolet
decomposition, volatilization at $50^{\circ}C$, and to storage for two
sols at $10^{\circ}C$. Although we report on a mixture of gamma Fe_2O_3
and silica sand that stimulated the LR nutrient reaction with
hydrogen peroxide and reduced the rate of hydrogen decomposition
under various storage conditions, the Mars analog soil prepared
by the Viking Inorganic Analysis Team to match the Mars analytical
data does not cause such effects. Nor is adequate resistance to
UV irradiation shown.

On the basis of the results and consideration presented
herein, while the hydrogen peroxide theory remains the most, if
not only, attractive chemical explanation of the LR data, it
remains unconvincing on critical points. Until problems
concerning the formation and stabilization of hydrogen peroxide
on the surface of Mars can be overcome, adherence to the scientific
evidence requires serious consideration of the biological theory.

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II. Introduction

The Viking Labeled Release (LR) life detection experiment
on the surface of Mars produced data consistent with a
biological interpretation (1-3). In considering the plausibility
of this interpretation, terrestrial life forms have been
identified which could serve as models for Martian microbial
life (4) and analysis of the landscape at the Mars landing site
has indicated color and feature changes that may be attributable
to biological activity (5). However, because of the hostility
of the Martian environment to life as we know it and the failure
to find organics on the surface of Mars, several nonbiological
explanations have been advanced as alternate explanations of
the Viking LR data. Most of these nonbiological theories,
such as reactions caused by or with ultraviolet radiation,
gamma Fe_2O_3, metalloperoxides or superoxides, have been largely
discounted on the basis of our earlier investigations (4,6).
Reaction of the LR nutrient with putative surface hydrogen
peroxide remains the leading nonbiological candidate. Hydrogen
peroxide has been shown to react with the LR nutrient to produce
responses in some respects similar to those observed on Mars (4).

Unlike the biological possibility, the hydrogen peroxide
theory can be intensively examined on Earth under controlled
experimental conditions to determine its probability. In order
for hydrogen peroxide to account for the flight data, it must
fulfill the following four criteria:

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- It must produce an active flight-like response with the LR nutrient under simulated flight conditions.

- It must display thermal sensitivity to $160^{\circ}C$, $50^{\circ}C$ and to long-term storage at $10^{\circ}C$ similar to that observed (1-3) on flight.

- A mechanism must exist for its formation and accumulation in sufficient quantities to account for the magnitude of the flight response.

- A mechanism must exist for its stability on the Mars surface material even in the presence of intense ultraviolet radiation.

During this contract period, we have experimentally examined each of these issues in an attempt to develop the hydrogen peroxide explanation as far as possible. These data, their interpretations, and their adequacy in accounting for flight data from the Viking Labeled Release experiment are presented in detail herein, evaluated, and briefly compared to the adequacy of a biological explanation for the LR data.

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III. <u>Reactivity of Hydrogen Peroxide with Labeled Release Nutrient</u>

Experiments have been conducted in the LR Test Standards Module (TSM), an instrument replicating flight configuration in all essential components (7), to determine whether hydrogen peroxide can mimic the Mars active agent by producing a positive LR response upon addition of the LR nutrient. In these experiments, 0.5 cc hydrogen peroxide were added to the LR TSM test cell and equilibrated for several hours at $10^{\circ}C$ under 5 torr carbon dioxide; the hydrogen peroxide concentration was such that the final concentration would be $10^{-1}M$ after the addition of 0.1 ml nutrient. The results following the addition of VM1 are reproduced from an earlier report (4) in Figure 1. As shown, both the kinetics and magnitude of the subsequent ^{14}C-labeled gas evolution are similar (but not identical) to those observed during the Viking mission. Upon injection of additional nutrient, after approximately 120 hours of incubation, a brief spike precedes a faster rate of gas evolution. Although the spike resembles flight data, the subsequent increased response contrasts sharply with the absorption of headspace gas observed in flight data. In the TSM, nutrient appeared to limit the TSM response following first nutrient injection. This is in contrast to the flight data in which the active Mars agent appears to be absent at the time of the second injection.

Figure 1

REACTION BETWEEN H_2O_2 (10^{-1}M) AND VM1 IN LR TSM
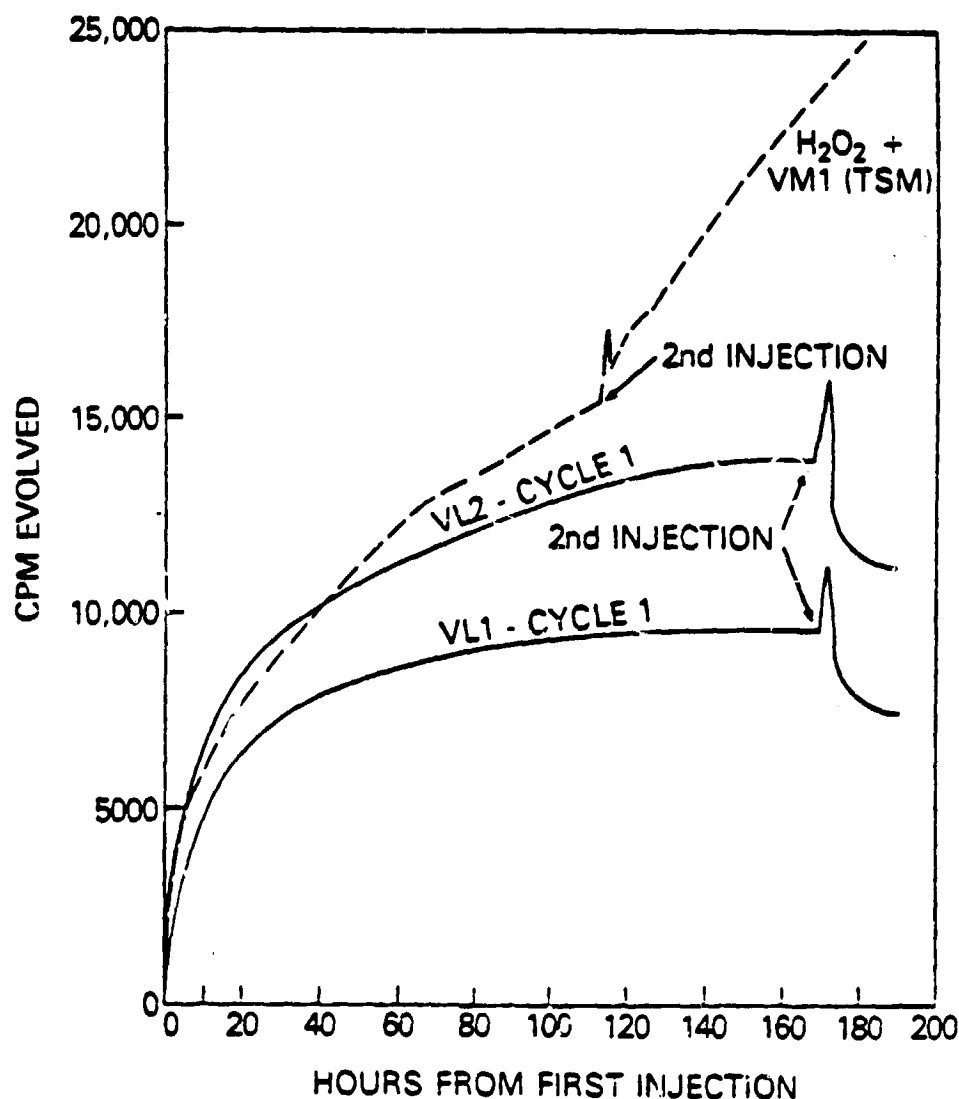
A 0.5 cc aliquot of 1.2×10^{-1}M hydrogen peroxide was adjusted to pH 6.4 and added to the TSM test cell. After equilibration in the sealed test cell at 10°C under 6 torr carbon dioxide, 0.115 ml of VM1 was injected according to the flight sequence. A second nutrient injection was added after approximately 113 hours of incubation. Evolved radioactivity (---) is compared to that obtained from the first LR cycle on each of the Viking landers.

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Although hydrogen peroxide at a concentration of 10^{-1}M can react with the LR nutrient in a flight-like instrument to produce a flight-like active response, it is not likely that hydrogen peroxide could be present on the surface of Mars at such high concentrations. However, it is known from previous work (4) that the hydrogen peroxide reaction with LR nutrient is stimulated by metal catalysts. Thus, in the presence of Mars metal catalysts, it may be possible to produce a response of the magnitude observed in Figure 1 with lesser amounts of hydrogen peroxide.

Figure 2 presents the results of experiments designed to determine how far the hydrogen peroxide concentration can be lowered in the presence of a metal catalyst and still produce a response of flight magnitude. Because time constraints preclude use of the TSM, these experiments were performed by the getter technique. Thus, hydrogen peroxide was added to a 0.5 cc sample of a Mars analog soil contained in a sterile screw cap vial. The soil selected was a mixture of gamma Fe_2O_3 and silica sand (15/85%) on which hydrogen peroxide was found to be stable (see later). After equilibration in a glove box at room temperature under 760 mm nitrogen, 0.1 ml of LR nutrient was added to each test vial and the vials immediately capped with a $Ba(OH)_2$-soaked getter pad. Getter pads were changed at intervals, dried under an infrared lamp, and counted for evolved radioactivity in a Nuclear Chicago gas flow counter. Counting

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Figure 2

**Effect of H_2O_2 Concentration on Reaction with VMI
in Presence of γFe_2O_3/Silica Sand (15/85%) Mixture**
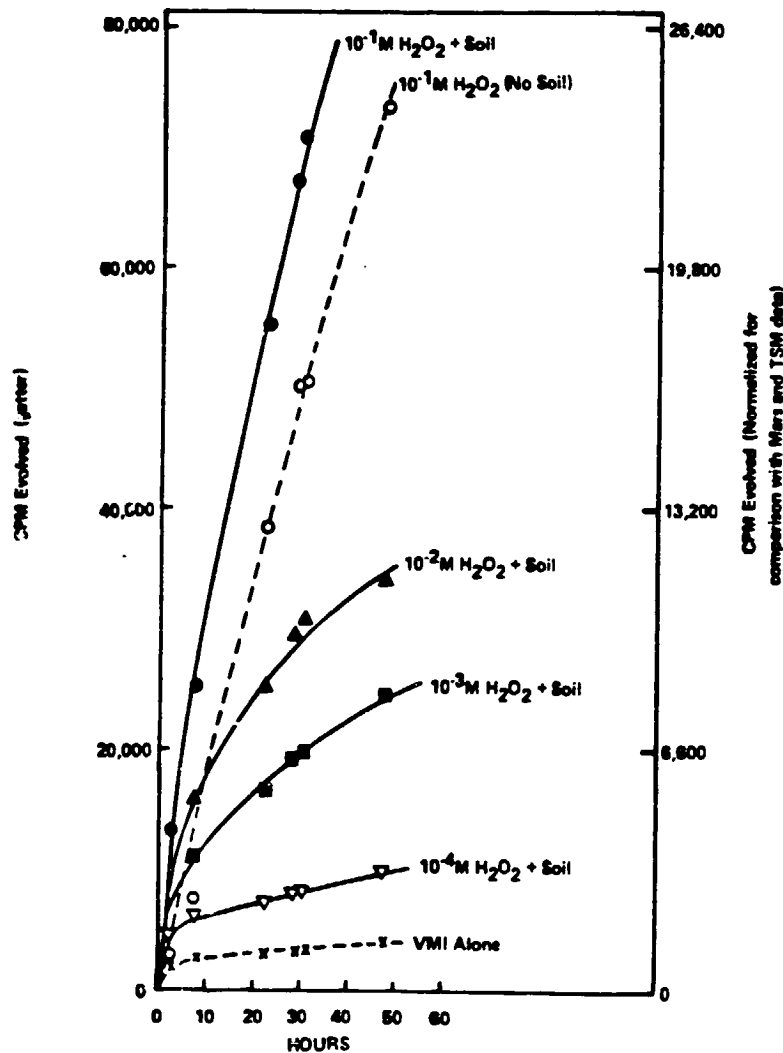
Screw cap vials containing 0.5 cc of a mixture of gamma Fe_2O_3/silica
sand (15/85%) were placed in a glove box equilibrated under one
atmosphere of nitrogen at room temperature and 0.22 ml of a hydrogen
peroxide solution was added to each vial. Immediately thereafter,
each vial received 0.11 ml of the LR nutrient. The concentration of
hydrogen peroxide in each vial was such that after the nutrient
addition the final concentration would be 10^{-1}M (●—●), 10^{-2}M (▲—▲),
10^{-3}M (■—■), or 10^{-4}M (▽—▽), as indicated. Evolved radioactive gas
was collected on $Ba(OH)_2$-soaked getter pads which were changed at
various intervals and counted by the gas flow technique at approxi-
mately 10% counting efficiency. Results are compared to those ob-
tained from LR nutrient and 10^{-1}M hydrogen peroxide in the absence
of soil (o--o) and from nutrient alone (x---x). All reactions were
conducted in duplicate. The scale on the left gives raw data obtained
whereas the scale on the right gives normalized counts had the
experiment been conducted either in the TSM or in a flight instrument.

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efficiency was estimated at 10% versus the 3% counting efficiency of the solid state beta detectors in the TSM and flight instruments.

Results obtained by mixing LR nutrient, gamma Fe_2O_3, and varying concentrations of hydrogen peroxide are compared in Figure 2 to a control in which LR nutrient was added to 10^{-1}M (final concentration) hydrogen peroxide in the absence of a metal-containing soil. Because the conditions of this control are similar to the same control used in Figure 1, this control permits approximate normalization of Figure 2 data to the data presented in Figure 1 which was obtained with the TSM and with the flight instruments. (It should be noted that differences in kinetics between Figures 1 and 2 are in part attributed to differences in test cell geometry between the two systems and in part to the fact that evolved radioactive gas in the TSM remains in equilibrium with the soil whereas it is removed from the headspace in a getter experiment.)

The results in Figure 2 show that the metal catalyst (gamma Fe_2O_3) significantly stimulates the reaction between hydrogen peroxide and the LR nutrient. Although difficult to quantitate the relationship between results in Figures 1 and 2, it nonetheless appears that the hydrogen peroxide concentration can be lowered to the 10^{-3} to 10^{-2}M range in the presence of a metal catalyst and still produce a positive response of flight magnitude with LR nutrient. Further, for all concentrations of

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hydrogen peroxide, the kinetics of gas evolution in the presence of the metal catalyst are initially faster and then slower than those observed with 10^{-1}M hydrogen peroxide in the absence of a metal catalyst. This shape was also observed with flight data (see Figure 1). It is concluded that, in the presence of a suitable metal catalyst, hydrogen peroxide can fulfill the first criteria of the Mars active agent, namely, that of mimicking the LR active response in the presence of a metal catalyst.

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IV. Thermal Sensitivity of Hydrogen Peroxide in the TSM

Labeled Release data returned from Mars showed that the active response with the LR nutrient was obliterated by pretreatment of the Mars surface material at $160^{\circ}C$ for three hours. The active agent was also partially destroyed by heat treatment for three hours at $50^{\circ}C$, a finding that gave strong support to the biological interpretation. In order for hydrogen peroxide to be the active agent on Mars, it must show similar patterns of thermal sensitivity.

A. Liquid Hydrogen Peroxide

Hydrogen peroxide is known (8,9) to be decomposed at $160^{\circ}C$ although it would be stable to the thermal regime at $50^{\circ}C$. This has been verified by our previous experimental results (4) in which hydrogen peroxide stability to various pretreatments was measured by its subsequent ability to react with the LR nutrient in a getter-type experiment. Under 10 torr nitrogen, hydrogen peroxide was destroyed by three hours heating at $160^{\circ}C$ but not by three hours heating at $50^{\circ}C$. However, in the presence of a metal catalyst such as gamma Fe_2O_3, hydrogen peroxide could be either partially or totally decomposed at $50^{\circ}C$, depending on the amount of iron present with the hydrogen peroxide during the heat treatment. From these results, it was initially concluded that hydrogen peroxide could meet the thermal criteria established for the active agent in the Mars surface material.

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Similar studies have been conducted this year in
the LR TSM in order to take into account any peculiarities of
the flight instrument. In these experiments, liquid hydrogen
peroxide was added to the LR TSM test cell and equilibrated at
5 torr carbon dioxide. The test cell was then sealed and, as
appropriate, the hydrogen peroxide subjected to a thermal
pretreatment essentially identical to that conducted on flight.
After then cooling to $10^{\circ}C$ and equilibrating at that temperature
for several hours, LR nutrient was injected into the test cell
via the flight injection sequence (7) and the hydrogen peroxide
activity present was monitored by the amount of radioactive
carbon dioxide subsequently evolved. As in the flight instrument,
radioactive gas was counted at 3% efficiency.

The results of the hydrogen peroxide thermal sensitivity
studies in the TSM are presented in Figure 3. As shown, pre-
treatment for three hours at $160^{\circ}C$, $50^{\circ}C$, and even $40^{\circ}C$,
essentially destroys or renders hydrogen peroxide unavailable
for reaction with the LR nutrient. Some unexplained residual
gas evolution occurred later in the time course following nutrient
injection onto a $160^{\circ}C$ treated sample.

This unexpected sensitivity of hydrogen peroxide to heat
treatment in the TSM, reported previously (4), has been the
subject of considerable investigation during this contract period.

Figure 3

**EFFECT OF PRE-HEATING H₂O₂
ON REACTION BETWEEN H₂O₂ AND VM1
IN LR TSM**
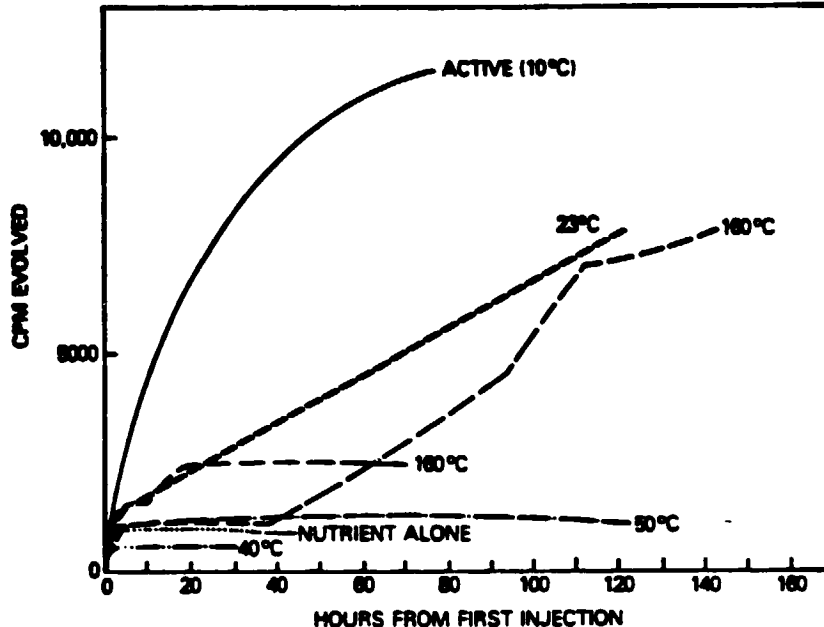
A 0.5 cc aliquot of 1.2×10^{-1} M hydrogen peroxide was adjusted to pH 6.4 (same pH as LR nutrient pH) and added to the TSM test cell. After equilibration in the sealed test cell at $10°C$ under 6 torr carbon dioxide, the sample was heated for 3 hours at $160°C$ (---), $50°C$ (-·-), $40°C$ (-··-), or $23°C$ (←--→), as indicated. After cooling and venting for 4 minutes through S/52, 0.115 ml of VM1 was added to the test cell according to the flight sequence. Evolved radio-activity is compared to that obtained from an unheated sample (———) and from nutrient alone (·····).

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Thus, the results have been extended (Figure 3) to show partial

reduction (approximately 50%) of subsequent hydrogen peroxide

activity with LR nutrient following three hours exposure

of hydrogen peroxide to temperatures as low as 23^OC. This

extreme temperature sensitivity in the TSM is inconsistent

with known properties of hydrogen peroxide (8,9) and with our

previous results (4) and suggests that hydrogen peroxide in

the flight instrument would be too temperature sensitive to be

the active agent which produced the LR results on Mars.

In pursuing an explanation for the enhanced thermal

sensitivity of hydrogen peroxide in the TSM, the hypothesis

that the test cell material (stainless steel) may catalyze the

thermal decomposition has been considered. However, in a

separate experiment, hydrogen peroxide was added to each of four

test cells. One was the TSM stainless steel test cell and the

other three were of identical size and configuration but composed

of glass (made for special-purpose TSM studies). The test

cells were sealed with Parafilm under one atmosphere air; glass

cells were maintained for three hours at 10°, 23°, or 40^OC, whereas

the stainless steel cell was maintained for three hours at 40^OC.

After the cells equilibrated at room temperature, the Parafilm

was removed and LR nutrient added to the pretreated hydrogen

peroxide. Evolved radioactive gas was collected with barium

hydroxide-soaked getter pads which were then dried and counted

by liquid scintillation. The results showed that hydrogen

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peroxide activity was unimpaired in each test cell, demonstrating
that the material comprising the TSM test cell was not
responsible for the enhanced thermal sensitivity observed during
the flight sequences for heat treatment. In confirmation of this
conclusion, a large volume of hydrogen peroxide was heated in
the TSM test cell for three hours at $50^{O}C$ according to the flight
sequence. After cooling to $10^{O}C$, the test cell was brought to
one atmosphere, opened, and the hydrogen peroxide removed by
pipette. A portion was then added to a glass cell and tested
for activity by adding LR nutrient and collecting evolved gas
by the getter technique. A second portion was added back into
the TSM test cell, equilibrated under 5 torr carbon dioxide, and
tested for activity with VM1 added by the normal nutrient injection
sequence. Both portions were fully active. The results show
that heat treatment per se at $50^{O}C$ in the TSM does not decompose
hydrogen peroxide. This is in accordance with published data
(8,9) on the thermal sensitivity of hydrogen peroxide.

A possible explanation for these observations is that
during the heat treatment, hydrogen peroxide was volatilized
out of the test cell to a colder part of the LR TSM. Candidate
areas include valve seats (e.g., S/52) in the head-end of the
test cell and the long swanneck tube leading to the solid state
detectors. (Detailed descriptions of the TSM instrument have been
presented earlier (7).) By the time nutrient was injected,

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hydrogen peroxide was no longer present in the test cell
and the resulting gas evolution pattern was typical of a nutrient-
alone response. However, this explanation appeared tentative
in that, at the end of each TSM experiment, the test cell had
been opened and the volume contained therein was measured.
Approximately 70 - 80% of the calculated total volume
(0.5 ml hydrogen peroxide plus 0.1 ml nutrient) was routinely
recovered and shown to be radioactive, thereby confirming that
injection had occurred.

It was next theorized that, perhaps, at the end of
the TSM experiment, the sequence of operations for opening the
test cell may have driven volatilized hydrogen peroxide back
into the test cell where it then recondensed.

A series of experiments was conducted in the TSM to
test this hypothesis. In these experiments, 0.5 ml hydrogen
peroxide was added to the test cell and heated to $50^{\circ}C$ under
Mars-like conditions according to the flight sequence. After
performing various operations in the sequence, the test cell
could be opened for observations of liquid present. This was
accomplsihed under Mars conditions by dropping the test cell
and observing it through the glass bell jar, or by opening the
test cell after bringing it and the bell jar to atmospheric
pressure, removing the bell jar, and measuring the liquid present
by pipette. The significant findings from many experiments
are summarized below (refer to Figure 4 for valve location):

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Figure 4

SCHEMATIC OF THE LABELED RELEASE EXPERIMENT
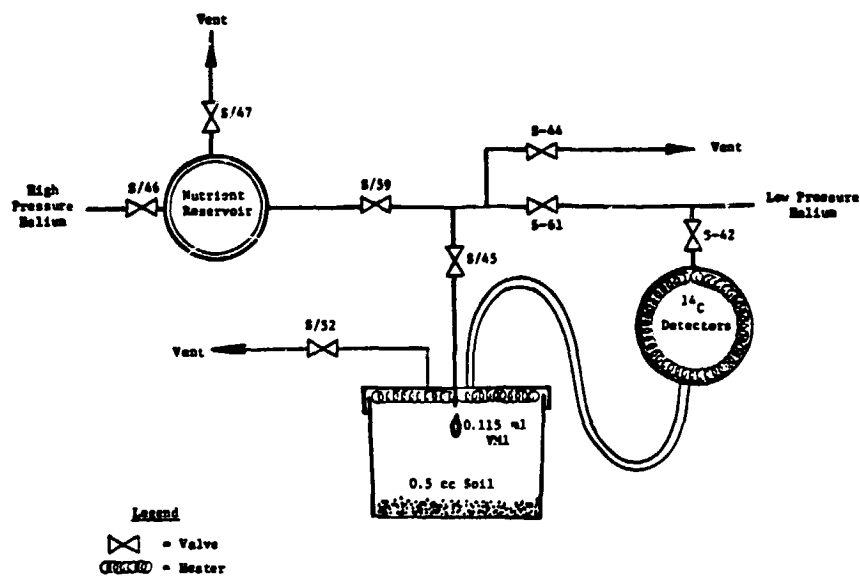
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1. If after cooling to $10^{\circ}C$, the test cell is vented out through S/52 to Mars atmosphere, and then both bell jar and test cell (via S/52) are simultaneously raised to one atmosphere, almost full volume is recovered upon opening the test cell.

2. If after cooling to $10^{\circ}C$, the test cell venting to Mars pressure through S/52 is omitted, and both bell jar and test cell pressures are raised to one atmosphere together through S/52 prior to opening the cell, no liquid is recovered.

3. If the test cell is lowered while heating at $50^{\circ}C$, no liquid is observed in the test cell. If the test cell is then closed and cooled to $10^{\circ}C$, and the bell jar pressure lowered, reopening the test cell "pulls" liquid out of the swanneck and the S/52 valve seat back into the test cell.

4. If foil is placed across the head-end, thereby preventing volatilization into other TSM regions during heating, all of the liquid is recovered after the sequence. Without the foil, the best recovery is about 70-80%.

5. Lowering the test cell after the S/52 vent to Mars pressure results in only partial recovery at this stage. Some liquid is found in the test cell and some is apparent around the openings into the test cell.

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These results confirm the hypothesis that hydrogen
peroxide is volatilized out of the test cell during 50°C heat
sterilization and returns to the test cell only after the cell
is vented through S/52 and opened to atmospheric pressure.
However, it should be noted that, because these experiments
deviate from the flight sequence whenever the test cell is opened
or the pressure changed, some extrapolation is necessary. Thus,
it has not been definitely established that any liquid, actually
drops into the test cell after venting through S/52. Study of
similar phenomena following heating at 160° is complicated by the
fact that the test cell cannot be lowered without briefly heating
to prevent damage to the seal. However, that no such re-entry
of liquid hydrogen peroxide occurs is indicated by the kinetic
data following nutrient injection (Figure 3), although the
late residual gas evolution after the 160°C sterilizations could
reflect small re-entries into the test cell.

In considering the implications of these findings to
flight data, it should be noted that in the flight sequence, heating
at either 160°C or 50°C is followed by cooling to 10°C and a four
minute vent to Mars atmosphere through S/52 prior to nutrient
injection. If the results obtained in the TSM are applicable
to the flight instrument, then hydrogen peroxide would have been
totally removed from the flight test cell in the heat sterilization
portion of the process. Venting through S/52 may have "pulled"
and condensed hydrogen peroxide onto the head end area rather
than into the test cell bottom where it could have reacted with
the LR nutrient.

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Thus, by this analysis, hydrogen peroxide cannot account for the Mars active agent unless it is complexed with the Mars soil in such a manner as to stabilize it to volatilization.

The applicability of these conclusions to the LR flight data is dependent upon the degree of similarity of the TSM configuration to that of the flight instrument. On the LR flight instrument, the main test cell heater (H-21) is contained in the head end assembly and a "trim" heater is located in the valve block. Both heaters must be used to achieve $160^{\circ}C$ sterilization temperatures although the trim heater alone was used on flight to obtain the $50^{\circ}C$ sterilization. The flight instrument also contains a detector heater (H-25) which is heated to approximately $110^{\circ}C$ during sterilization. The 13 inch swan-neck line between the test cell and the detector is unheated but is probably at the temperature of the LR module (approximately $10^{\circ}C$) although it could increase somewhat during sterilization due to conduction from the head end and detector heaters.

The major difference between the flight instrument and the TSM is in the heaters. In all other respects, the TSM is essentially identical to the flight instrument. The TSM has both a detector heater and a head end heater, similar to those on flight. In addition, there is also an auxiliary heater strapped around the test cell. To obtain sterilization temperatures of $160^{\circ}C$, both the auxiliary heater and the head end heater are used

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along with the detector heater. For the 50°C heating on the

TSM, only the head end heater was used (along with the detector

heater). The line between the test cell and the detector

is at room temperature (approximately 23°C) although it is

subject to heating by conduction during sterilization. The

information regarding the differences between the flight in-

strument and the TSM thermal patterns during the 50°C heating

is summarized in Table I. The major difference is that on the

flight instrument, the valve block temperature during the heating

cycle is higher than the head end whereas the reverse is true

in the TSM experiments. In considering the importance of this

difference, we located an obsolete TSM trim heater on the back

of the underside of the TSM valve block. Consultation with TSM

engineers (10) established that the location, although not

identical with that of the flight instrument, closely approximated

it. After connecting this heater, an experiment was performed

in which only the TSM valve block heater was used to obtain

temperatures of 50°C. These experiments confirm that the hydrogen

peroxide originally contained in the test cell had disappeared

or lost all activity by the time VM1 was subsequently injected

in the flight injection sequence. It is concluded that the

experiments conducted in the TSM with the auxiliary heater

showing hydrogen peroxide volatilization are directly applicable

to the flight instrument. This analysis would predict that no

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TABLE I

INSTRUMENT TEMPERATURES AT INDICATED LOCATIONS DURING 50°C HEAT REGIME

Area	TSM	Viking Lander 2 Cycle 2	Viking Lander 2 Cycle 4
Valve Block	< 55°C	> 62°C	> 55°C
Head End	55°C	62°C	55°C
Test Cell Bottom	~ 50°C	~ 51°C	~ 46°C
Detectors	110°C	110°C	110°C
Swanneck	>23°C	> 10°C	> 10°C

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hydrogen peroxide could have remained in the LR test cells on

Mars after the $50^{\circ}C$ heatings to explain the responses obtained.

B. Effect of Various Mars Analog Soils on Hydrogen Peroxide
    Stability to $50^{\circ}C$

The complete volatilization of hydrogen peroxide out

of the TSM test cell during the $50^{\circ}C$ heating regime (see preceding

section) would be expected to occur in the Viking instrument on

Mars. However, to press the matter further, it was theorized

that some mechanism exists on Mars to stabilize hydrogen peroxide

to volatilization at $50^{\circ}C$ while partially decomposing it through

metal catalysis. The only apparent mechanism would be formation

of a complex between hydrogen peroxide and the Mars surface material

(which contains approximately 18% iron oxide).

Experiments conducted this quarter have examined the

ability of gamma Fe_2O_3 and silica sand (analytically determined

to be present on Mars, reference 11), to stabilize hydrogen

peroxide. In each experiment, 0.5 cc of soil sample were placed

in the TSM test cell and 0.25 ml of hydrogen peroxide added (final

concentration after nutrient addition = $10^{-1}M$). The test cell

was sealed and the mixture equilibrated at $10^{\circ}C$ under 5 torr

carbon dioxide. As appropriate, the test cell was then heated

for three hours at $50^{\circ}C$ and then re-cooled to $10^{\circ}C$ prior to

nutrient addition; control samples were incubated for an equal

period of time at $10^{\circ}C$ prior to nutrient addition. LR nutrient

(0.115 ml) was then injected onto the mixture via the normal

flight injection sequence and the evolved ^{14}C-labeled gas was

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followed as a monitor of hydrogen peroxide activity. Counting

efficiency in the TSM is approximately 3%.

Initial experiments were conducted with samples of

pure silica sand (pH 6.9) or with pure gamma Fe_2O_3 (pH 3.0).

(Separate experiments reported in later sections had already

established that hydrogen peroxide was stable at room temperature

for three hours on both samples and that neither sample showed

biological activity with LR nutrient alone.) As shown in Figure

5, hydrogen peroxide was stable on both samples for the nine

hour incubation period at $10^{\circ}C$; silica sand had essentially no

effect on subsequent reactivity with the LR nutrient whereas

the iron sample significantly stimulated the reaction, as reported

earlier (4). However, the $50^{\circ}C$ heat treatment of hydrogen

peroxide in contact with either sample resulted in total loss

of all activity upon subsequent addition of the LR nutrient,

indicating that neither sample had stabilized hydrogen peroxide

to volatilization during heating.

The ambient Mars temperature suggested yet a further

attempt to generate a heat-stable complex between hydrogen

peroxide and a Mars analog soil. Accordingly, experiments were

conducted in which hydrogen peroxide was added onto the soil

sample frozen at $-30^{\circ}C$. A liquid nitrogen bath was placed around

the TSM test cell to accomplish the cooling. Hydrogen peroxide

added to a soil sample contained in the cooled TSM test cell froze

instantly upon contact. The test cell was then held at $-30^{\circ}C$

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Figure 5

Effect of γFe_2O_3 and of Silica Sand
on 50°C Volatilization of H_2O_2 in TSM
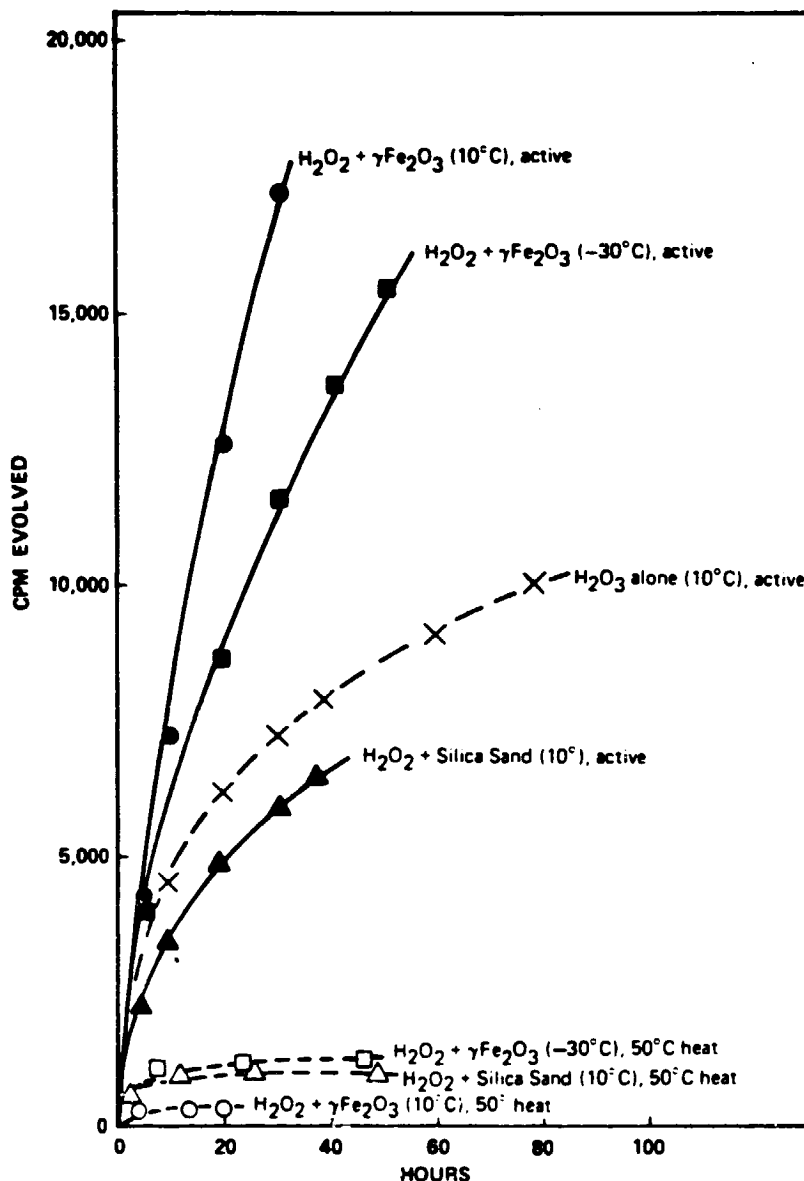
Samples of 0.5cc of either γFe_2O_3 or of silica sand were added to the TSM test cell under an atmosphere of carbon dioxide. After sample equilibration at either 10°C or at -30°C, as indicated in parentheses, 0.3 ml of a 1.4×10^{-1} M hydrogen peroxide solution (pH 6.5) was added. The test cell was quickly sealed under 5 torr carbon dioxide and incubation continued for an additional three hours at either 10°C or -30°C. All samples designated for "active" sequences were then maintained at 10°C for an additional six hours whereas samples designated for "sterile" sequences were heated to 50°C for three hours and then cooled to 10°C for an additional three hours. After the pre-incubation regime, 0.115 ml of LR nutrient was injected via the flight sequence and subsequently evolved radioactivity monitored. Assuming complete mixing of nutrient and hydrogen peroxide, the final hydrogen peroxide concentration was 10^{-1} M. The results with γFe_2O_3 or with silica sand are compared to those obtained with 10^{-1} M hydrogen peroxide alone (no soil) after a nine hour pre-incubation at 10°C. All data have been corrected for background counts.

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for an additional three hours after which the temperature was raised to $10^{\circ}C$ with the test cell sealed under 5 torr carbon dioxide. The sample was then heated for three hours at $50^{\circ}C$ according to the "cold sterilization" flight regime. After again cooling the sample to $10^{\circ}C$, LR nutrient was added via the flight injection sequence. A control similarly treated at $-30^{\circ}C$ was maintained at $10^{\circ}C$ for a time period comparable to that used during the $50^{\circ}C$ regime. The results obtained with pure gamma Fe_2O_3 (Figure 5) indicate that freezing had no appreciable effect on hydrogen peroxide activity with the LR nutrient; further, no complex formation occurred to stabilize hydrogen peroxide to volatilization at the $50^{\circ}C$ treatment.

The possibility that complex formation with hydrogen peroxide could require an analog soil that contains both silica and a metal component was then examined using a mixture of 15% gamma Fe_2O_3 and 85% silica sand. Hydrogen peroxide was added to 0.5 cc samples of this mixture contained in the TSM test cell and pre-equilibrated at either $10^{\circ}C$ or at $-30^{\circ}C$. For each pre-equilibration temperature, samples were also examined with and without subsequent heat treatment for three hours at $50^{\circ}C$. The results of these experiments are shown in Figure 6. As shown, for samples not heated at $50^{\circ}C$, hydrogen peroxide pre-equilibrated at either $10^{\circ}C$ or at $-30^{\circ}C$ with the iron/silica sand mixture reacted when LR nutrient was added. (The lower

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Figure 6

Effect of γFe_2O_3/Silica Sand Mixture (15/85%) on 50°C Volatilization of H_2O_2 in TSM
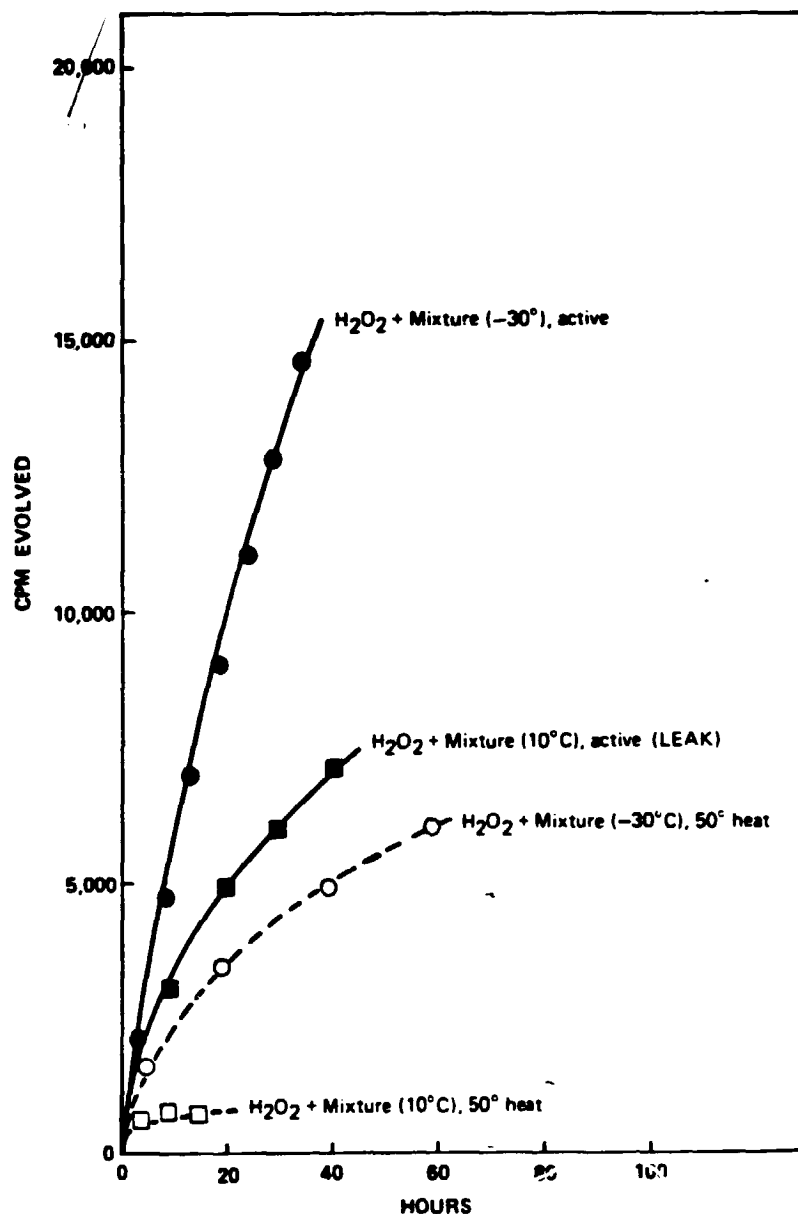
Experimental conditions and procedures are identical to those described for Figure 1 except that the 0.5cc sample consisted of a mixture of 15% γ Fe_2O_3 and 85% silica sand. One run, as indicated, had a test cell leak although the magnitude of the leak could not be determined. The results obtained are thus lower than would have been observed in the absence of this leak.

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reactivity of the sample pre-equilibrated at 10^OC reflects an
observed leak in the test cell.) For samples subjected to the
50^OC heat regime, a differential effect on hydrogen peroxide is
observed depending on the pre-equilibration temperature.
Following 10^OC pre-equilibration, no reactivity occurred with
the LR nutrient. However, for the -30^OC pre-equilibration, a
moderate level of reactivity was observed after the 50^OC heating.
This apparent inhibition of the active agent is reminiscent of
that observed on Mars. This result could only be obtained if
the sought-for complex between hydrogen peroxide and the iron/
silica sand mixture had, indeed, formed at -30^OC such that the
resulting complex was stabilized against subsequent volatilization
at 50^OC. The partial reactivity observed could reflect either
partial stabilization or total stabilization coupled with partial
decomposition by metal catalysis. Thus, if soil composition
and conditions on Mars permit this mechanism to operate, and if
sufficient quantities of hydrogen peroxide accumulate on Mars,
hydrogen peroxide could be responsible for the 50^OC heating
response as well as for the active and the 160^OC sterilization
response obtained on Mars.

In an effort to gain further support for this concept,
similar experiments have been conducted in the TSM with the
Mars analog soil B2. This soil was prepared by the Viking
Inorganic Analysis Team to match the analytical spectra of

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the Mars surface material (11) containing approximately 42%

SiO_2 and 17% iron oxide, approximately 11% of which is gamma

Fe_2O_3 (12). The B2 analog soil was shown to be biologically

sterile by adding LR nutrient to a 0.5 cc sample contained in

the TSM test cell. Subsequent experiments were conducted with

hydrogen peroxide added to the B2 soil and pre-equilibrated at

$-30^{O}C$. However, as had been found earlier (4) in experiments

in which hydrogen peroxide was added to the B2 soil at room

temperature, contact with the frozen sample resulted in rapid

decomposition of hydrogen peroxide. Thus, if this model of the

Mars soil is correct, any hydrogen peroxide present on Mars

could not have been stabilized by the low temperature.

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V. Stability of Hydrogen Peroxide on Various Mars Analog
     Soils

    A. Room Temperature Studies

        Preliminary experiments reported earlier (4) indicated
that hydrogen peroxide may not be stable on the surface of Mars.
It was shown that three hours of contact with Mars Analog B2
or with a fine glass powder destroyed hydrogen peroxide. The fine
particle size of each analog soil (from 10-100 μ for the B2 soil
and from 50-200 μ for the glass powder) supports the suggestion
of Edwards (13) that hydrogen peroxide may not be stable on the
fine surface material of Mars. An alternate hypothesis was that
hydrogen peroxide may have been destroyed by the trace organic
matter present in the analog soils. However, TOC analyses
indicated that the organic content of the B2 soil and the glass
powder are 800 ppm and less than 30 ppm, respectively; the 30 ppm
is probably insufficient to account for destruction of all hydrogen
peroxide present. Thus, the organic contaminant hypothesis does
not appear consistent with the data. Attempts to remove organics
from the Mars Analog Soil B2 did not improve subsequent hydrogen
peroxide stability on those soils. Casting doubt on size theory,
however, is the finding (4) that hydrogen peroxide is stable to
incubation for three hours on gamma Fe_2O_3 despite its particle
size range of 10-100 μ.

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Although the preliminary results suggested that
hydrogen peroxide may not survive on Mars, the perplexing flight
data prompted a further investigation into the stability of
hydrogen peroxide on candidate Mars analog soils. In getter-
type experiments, 0.5 cc of the analog soil was added to a
sterile glass vial and equilibrated in a room temperature glove
box under a nitrogen atmosphere at 760 mm Hg. To each soil were
added 0.22 ml of a 1.5×10^{-1}M aqueous solution of hydrogen
peroxide (adjusted to approximately pH 6.5) followed, either
immediately or after three hours, by 0.11 ml of LR nutrient
(pH 6.4). After the nutrient addition, the vials were sealed and
evolved $^{14}CO_2$ was trapped with a $Ba(OH)_2^-$ soaked getter pad placed
inside the caps. At intervals, the pads were replaced with
fresh pads and the exposed pads dried and counted by the gas flow
technique (approximately 10% efficiency).

Six Mars analog soils were selected for stability
studies with hydrogen peroxide. Two of these samples were
prepared by the Viking Inorganic Analysis Team to match the Mars
inorganic spectra (11,12) whereas the other samples consisted of
pure gamma Fe_2O_3, pure silica, and a mixture of gamma Fe_2O_3 and
glass powder. The samples covered a wide range of particle sizes,
organic content, and pH values as follows:

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Soil	ppm organics	Particle Size	pH
Gamma Fe_2O_3 (Cobaloy X4107)	800	10-100 μ	3.0
Silica Sand	170	420-840 μ	6.8
Glass Powder	30	50-210 μ	9.9
γFe_2O_3 Glass Powder Mix (15/85%)	145*	10-210 μ	9.1
Mars Analog B2	600	10-100 μ	7.9

* Calculated (all other values determined by TOC)

Results with each of these soils are shown in Figures 7 and 8. As shown in Figure 7, hydrogen peroxide is stable for at least three hours at room temperature on silica sand and on gamma Fe_2O_3. Results with silica sand resemble those obtained in the absence of any soil whereas hydrogen peroxide activity with the LR nutrient is significantly stimulated in the presence of gamma Fe_2O_3. On the other hand, hydrogen peroxide is highly unstable on glass powder and shows intermediate stability with the iron/ glass powder mixture. With Mars Analog Soil B2 (Figure 8), a stimulatory effect is evident without pre-incubation although all hydrogen peroxide activity is essentially destroyed following three hours of incubation on the soil. In considering all the data in Figures 7 and 8, the stability of hydrogen peroxide did not appear to correlate with either organic content or with particle size although some correlation between stability and low pH became apparent.

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Figure 7

Effects of γFe_2O_3, Silica Sand and Glass Powder on H_2O_2 Stability
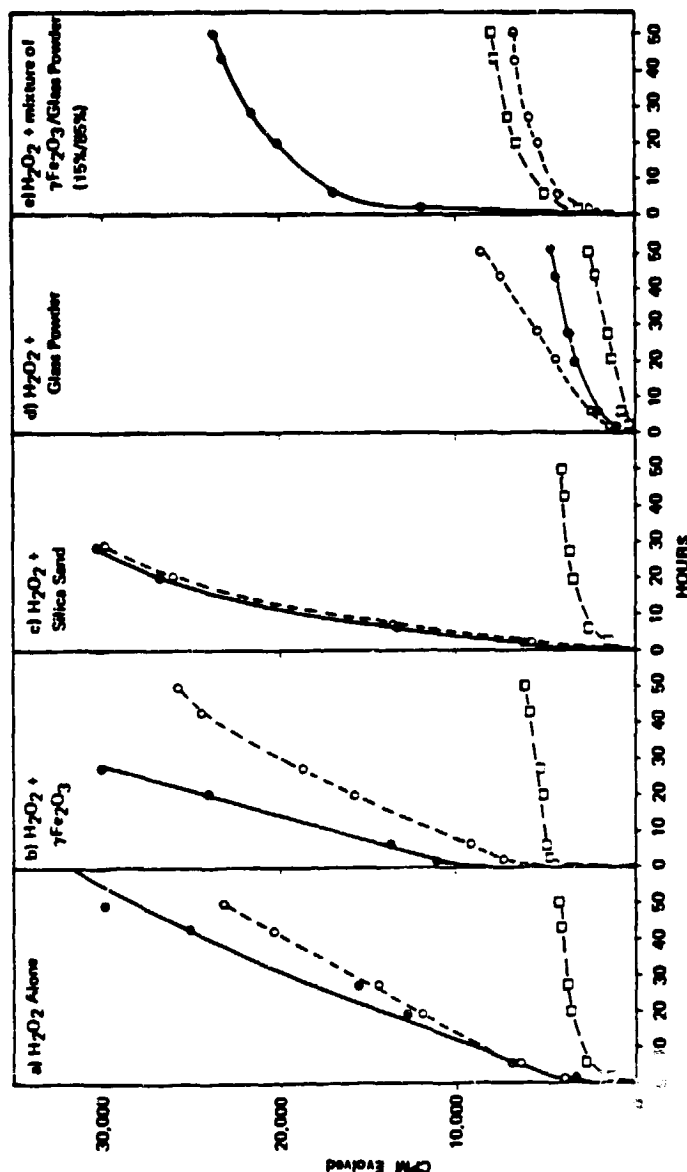
Sterile screw cap vials were prepared either (a) without a soil sample, or with a 0.4 g sample of
(b) pure gamma Fe_2O_3, (c) silica sand, (d) glass powder, or (e) a 15/85% mixture of gamma Fe_2O_3 and glass powder.
After placing the vials in a glove box equilibrated under one atmosphere of nitrogen at room temperature, 0.22 ml
of a solution of 1.5×10^{-1} M hydrogen peroxide (pH 6.5) was added to each vial. Either immediately (●——●) or
three hours after (o---o) the hydrogen peroxide addition, each vial then received 0.11 ml of the LR nutrient. For
each sample, a control was also conducted in which water was substituted for hydrogen peroxide as a monitor of
biological or nonbiological activity of the LR nutrient (□--□). Evolved radioactive gas was collected on
Ba(OH)$_2$ soaked getter pads which were changed at various intervals and counted by the gas flow technique at
approximately 10% counting efficiency. All reaction vials were conducted in duplicate.

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Figure 8

Effect of Mars Analog B2 on
Reaction Between VMI and H_2O_2
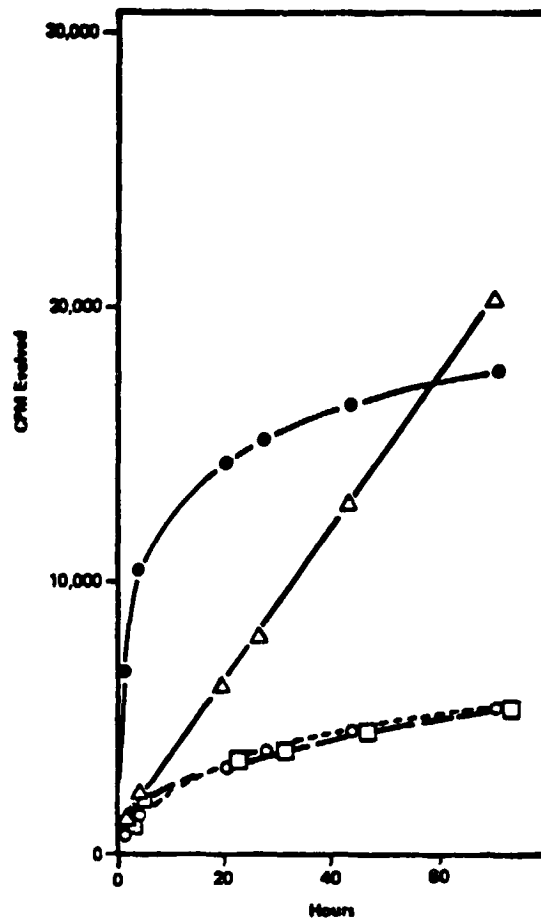
Sterile glass screw cap vials containing 0.4 g of Mars Analog B2 soil were placed in a glove box equilibrated under one atmosphere of nitrogen at room temperature and 0.22 ml of a solution of 1.5×10^{-1} M hydrogen peroxide (pH 6.5) was added to each vial. Either immediately (●——●) or three hours after (o---o) the hydrogen peroxide addition, each vial received 0.11 ml of the LR nutrient. Evolved radioactive gas was collected on $Ba(OH)_2$ soaked getter pads which were changed at various intervals and counted by the gas flow technique at approximately 10% counting efficiency. Results are compared to those obtained from LR nutrient and hydrogen peroxide in the absence of soil (△——△). A control was also conducted in which LR nutrient was added to soil in the absence of hydrogen peroxide (□--□) to monitor for biological or nonbiological activity of nutrient with soil. All reactions were conducted in duplicate.

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In pursuing the possible correlation between hydrogen peroxide stability and soil pH, we obtained five additional iron samples for comparison to the original gamma Fe_2O_3 sample. The six samples, listed in Table II, were found to span a broad range of pH values and include samples of either gamma or alpha iron. The additional five samples were obtained by courtesy of Dr. Robert Hargraves of Princeton University who commented that they "behave" differently and differ in pH depending upon their source (preparation) (14).

Results of stability studies in which hydrogen peroxide was incubated for zero or three hours with these samples prior to the addition of the LR nutrient are shown in Figure 9. All experiments were conducted at room temperature under nitrogen at 760 mm Hg. As shown, without pre-incubation, hydrogen peroxide is stable on all soils except that at the highest pH. On the (Mapico Brown 422 Maghemite, pH 8.7 sample, hydrogen peroxide loses activity immediately. For the other alkaline sample (Lepidocrocite, pH 8.0), hydrogen peroxide activity is retained without pre-incubation, but is totally lost after three hours of pre-incubation with the soil. All other samples show considerable activity upon addition of the LR nutrient after three hours of pre-incubation of hydrogen peroxide with the sample, although activity losses range from minor to major.

These results support the hypothesis that hydrogen peroxide stability or soil is favored by low pH. That more than

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TABLE II

IRON (GAMMA AND ALPHA) SAMPLES TESTED

$\gamma\, Fe_2O_3$	MO2230 Maghemite	pH 2.5
$\gamma\, Fe_2O_3$	MO4228 Maghemite	pH 2.5
$\gamma\, Fe_2O_3$*	Cobaloy x4107	pH 3.0
$\alpha\, FeO\cdot OH$	Goethite	pH 3.8
$\gamma\, FeO\cdot OH$	Lepidocrocite	pH 8.0
$\gamma\, Fe_2O_3$	Mapico Brown 422 Maghemite	pH 8.7

* Gamma iron obtained from Vance Oyama and used for all previous work.

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Figure 9

Effect of H₂O₂ Preincubation with Various Fe₂O₃ Samples on Subsequent Reaction Between VM1 and H₂O₂
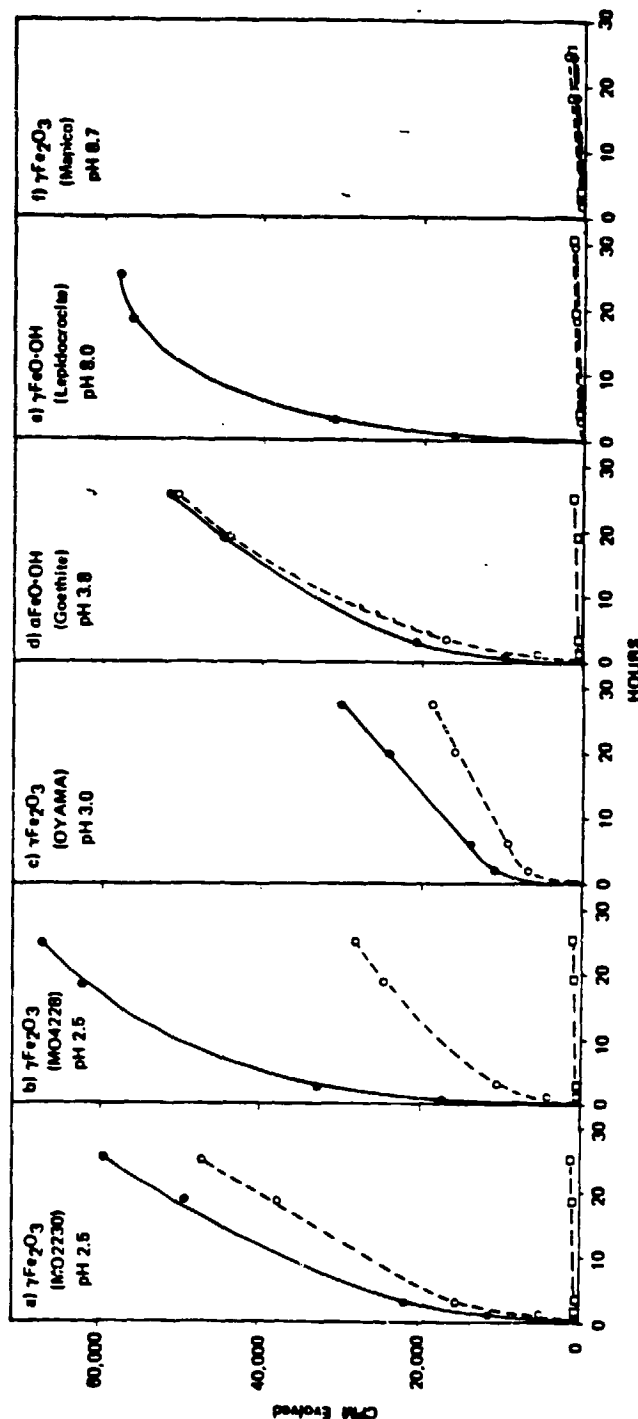
Sterile screw cap.vials were prepared containing 0.4 g of each of six gamma or alpha iron samples, as indicated. After placing the vials in a glove box equilibrated under one atmosphere of nitrogen at room temperature, 0.22 ml of a solution of 1.5 x 10⁻¹M hydrogen peroxide (pH 6.5) was added to each vial. Either immediately (●——●) or three hours after (o---o) the hydrogen peroxide addition, 0.11 ml of the LR nutrient was added to each vial. For each sample, a control was also conducted in which water was substituted for hydrogen peroxide as a monitor of biological or nonbiological activity of the LR nutrient (□---□). Evolved radioactive gas was collected on Ba(OH)₂ soaked getter pads which were changed at various intervals and counted by the gas flow technique at approximately 10% counting efficiency. All reaction vials were conducted in duplicate. Five of these soils were obtained from Dr. Hargraves and one was obtained from Mr. Oyama (as indicated). Soil pH was determined separately and is shown for each sample.

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pH alone is involved in stability is indicated by separate

studies in which aqueous solutions of hydrogen peroxide without

the soils were adjusted to varying pH values from pH 3 to 10

and LR nutrient added before and after three hours standing

at the indicated pH. The hydrogen peroxide solutions were found

to be stable and essentially no difference in stability was

found among solutions at different pH values. These findings

are consistent with published literature (8,9). This suggests

that some additional factor in the soil-metal composition is

present to catalyze hydrogen peroxide instability in high pH

soils or that the soil particulates will catalyze hydrogen

peroxide instability except when this effect is prevented by low

pH.

B. Effect of Low Temperature on Hydrogen Peroxide
    Stability with Soil

Studies were next undertaken to determine whether low

temperatures, in the presence and absence of ultraviolet light,

could enhance hydrogen peroxide stability on neutral or high

pH soils. (The effects of UV will be discussed in a later section).

The soils selected for these studies are Mars analog B2 soil

(pH 7.9) and the 15/85% mixture of gamma iron/silica powder

(pH 9.1). (Note that this is a different mixture than the gamma

iron/silica sand mixture used for Figure 2 and 6 data in previous

sections. Hydrogen peroxide is stable at room temperature on

the low pH silica sand mixture but not on the high pH silica

powder mixture.) All experiments were conducted by the getter

technique in which 0.5 cc of the analog soil was added to a

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sterile glass screw cap vial and the vial placed on dry ice
($<-70^\circ$C) contained within a glove box under nitrogen atmosphere
at 760 mm Hg. After temperature equilibration of the soil for
15 minutes, 0.22 ml of a 1.5×10^{-1}M solution of hydrogen
peroxide was added which instantly froze on the sample. The
vials were sealed and both vial and dry ice removed from the
glove box; after further incubation of the sample at $<-70^\circ$C for
zero or three hours, the vial was returned to the glove box,
the temperature raised to 10°C or 23°C, as appropriate, for
approximately 10 minutes and 0.11 ml of LR nutrient (pH 6.4)
added to the sample. The vials were quickly sealed and evolved
$^{14}CO_2$ was trapped by a $Ba(OH)_2$ getter pad placed in the vial cap.
At appropriate intervals, the pads were replaced with fresh pads
and the exposed pads dried and counted by the gas flow technique
(approximately 10% counting efficiency).

Results obtained with the gamma iron/silica powder
mixture are shown in Figure 10. In agreement with previous
data (Figure 6) this soil stimulates the reaction between hydrogen
peroxide and LR nutrient when both are added together onto the
soil sample at room temperature. Pre-incubation of hydrogen
peroxide on the soil for three hours at room temperature destroys
all subsequent activity with the LR nutrient. However, pre-
incubation on the soil at $<-70^\circ$C for either three or six hours
preserves most of the hydrogen peroxide activity as monitored
by subsequent nutrient addition. If, after incubation at $<-70^\circ$C

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Figure 10

Effect of Freezing ±Ultraviolet Radiation on Stability of H_2O_2 on γFe_2O_3/Silica Powder Mixture (15/85%)
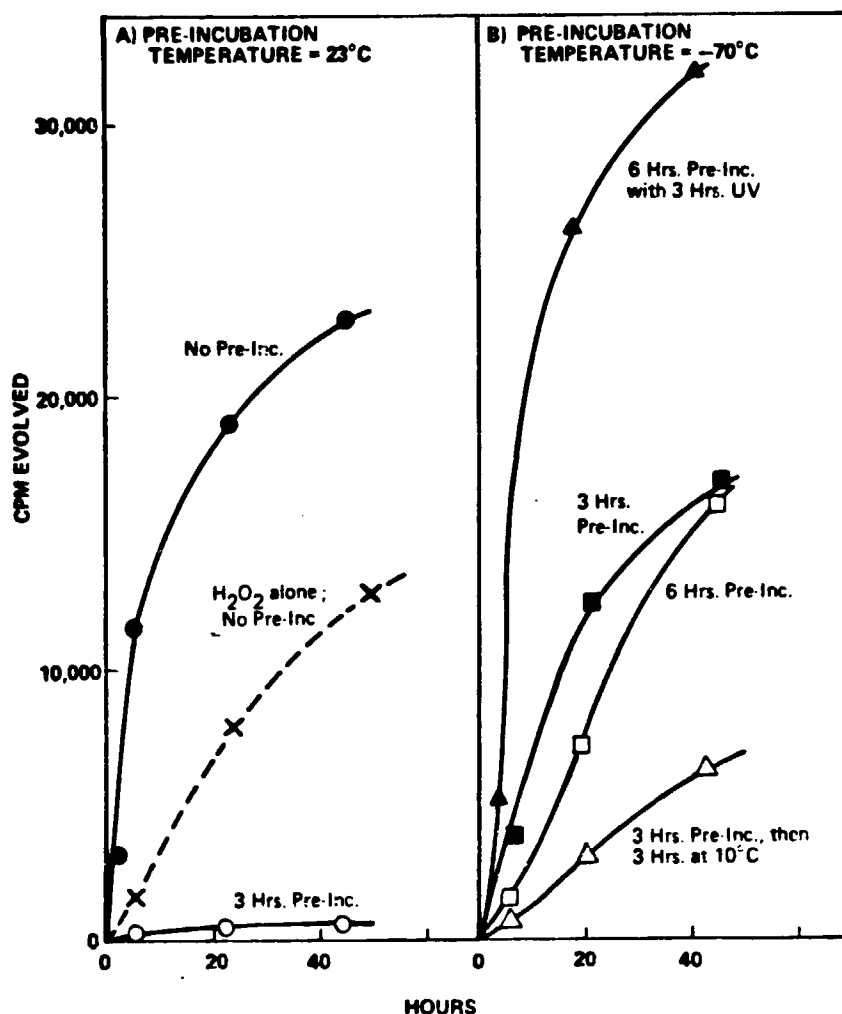
A mixture of 15% γFe_2O_3 and 85% silica powder was prepared and 0.5cc samples added to liquid scintillation vials. The vials were placed in a glove box under nitrogen at 760 mm Hg and equilibrated at either room temperature (A) or on dry ice at approximately -70°C (B). After temperature equilibration for 15 minutes, 0.22 ml of a 1.5×10^{-1}M hydrogen peroxide solution (pH 6.5) was added. For samples maintained at room temperature (A), 0.11 ml of LR nutrient was added with (o——o) and without (●——●) a three hour pre-incubation at room temperature. Reduced temperature pre-incubation regimes (B) were 3 hours at -70°C (■——■), 6 hours at -70°C (□——□), 3 hours at -70°C followed by 3 hours at 10°C (△——△), and 6 hours at -70°C with UV present (1620 $\mu W/cm^2$; 254 mμ) during the last three hours (▲——▲). Samples pre-incubated at reduced temperatures were raised to room temperature for approximately 10 minutes prior to nutrient addition. Radioactivity evolved following the nutrient addition was monitored by the getter technique using a Ba(OH)$_2$ soaked filter pad placed in the vial cap. Getter pads were changed at various intervals, dried, and counted in a gas flow counter at approximately 10% efficiency.

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for three hours, hydrogen peroxide is maintained at $10^{\circ}C$ for
an additional three hours prior to the nutrient addition, about
half of the hydrogen peroxide activity remains. With respect
to flight data, this suggests that little, if any, would be
expected to survive the two sol incubation at $10^{\circ}C$ prior to
nutrient addition.

Similar tests have been conducted with the Mars
analog B2 soil. As shown in Figure 11, pre-incubation of
hydrogen peroxide on B2 for three hours at room temperature
destroys all subsequent activity with the LR nutrient whereas
pre-incubation for three or six hours at $<-70^{\circ}C$ preserves most
activity. In contrast to the results obtained with the gamma
iron/silica powder mixture, however, three hours incubation at
$<-70^{\circ}C$ followed by an additional three hours at $10^{\circ}C$ essentially
destroys all activity.

These results suggest that, if a mechanism exists for
the formation of hydrogen peroxide on the surface of Mars, the
low ambient temperatures would contribute significantly to its
preservation on the surface of Mars. However, if the ambient
Mars temperature approaches $10^{\circ}C$, even briefly (i.e., three
hours), as might be expected at some latitudes during the
Martian summer, the peroxide would disappear from the surface.
Further, the minimum two day incubation period at $10^{\circ}C$ prior
to nutrient injection in the Mars LR experiment would have

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Figure 11

Effect of Freezing ±Ultraviolet Radiation on Stability of H_2O_2 on Mars Analog B2
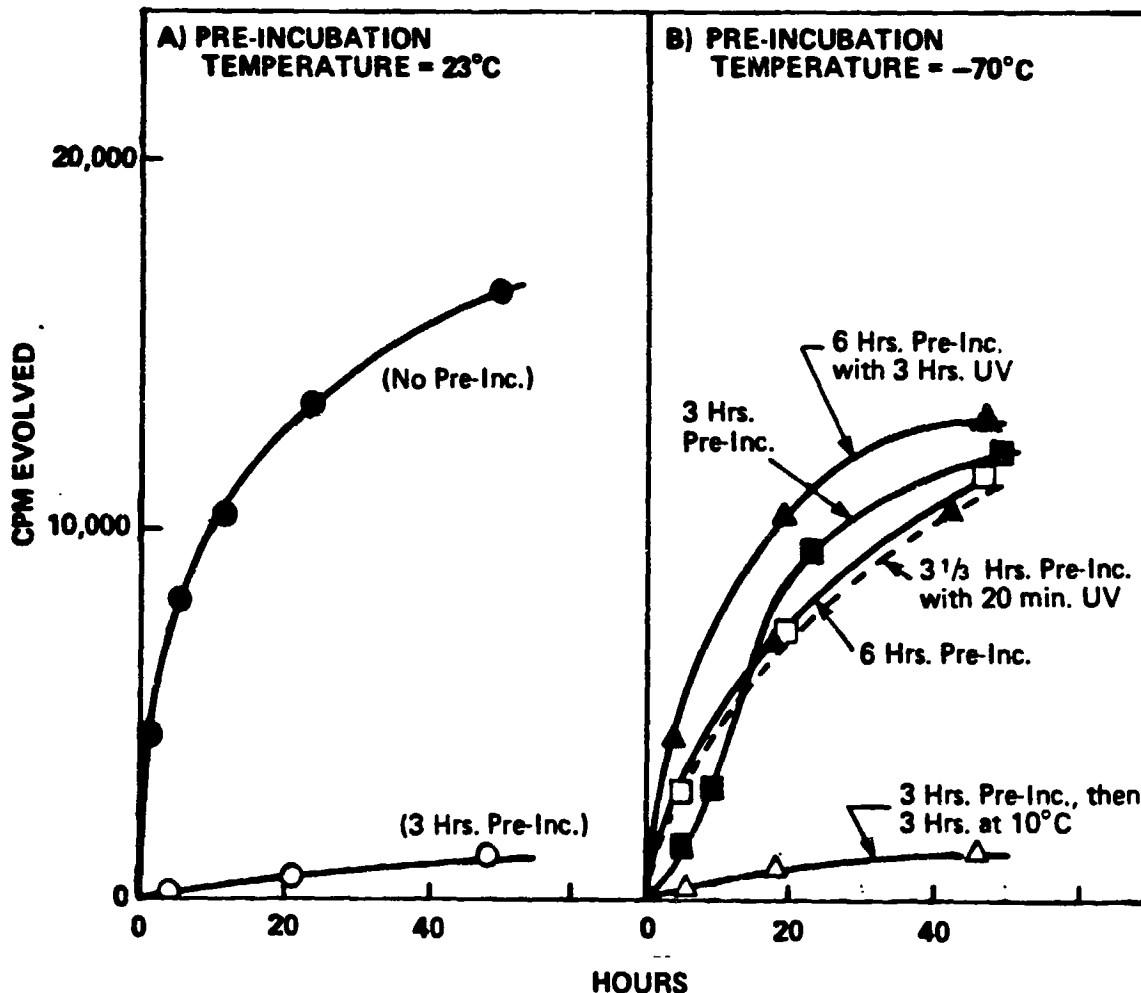
Experimental conditions and procedures are identical to those described for Figure 3 except that the 0.5cc sample consisted of Mars Analog Soil B2. One additional run was also conducted in which the sample was pre-incubated with hydrogen peroxide for 3 hours and 20 minutes at <-70°C with UV present during the last 20 minutes (▲- - -▲).

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eliminated reactivity of hydrogen peroxide if Mars soil is

composed as reported by the Inorganic Analysis experiment.

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C. <u>Effect of Flight Pre-Incubation Thermal Regime
    on Hydrogen Peroxide Stability</u>

In the flight experiments, the Mars surface temperature at the time of the various sample acquisitions ranged from $-21^{\circ}C$ to $-84^{\circ}C$ (3). After acquisition, Mars samples that later gave positive LR responses were placed into the R test cell and maintained at an average temperature of about $10^{\circ}C$ (diurnal range of approximately $9^{\circ}C$ to $14^{\circ}C$) for two sols prior to nutrient addition. The question next arises as to whether any hydrogen peroxide contained on the surface material could have remained stable under these storage conditions, particularly since the results in Figures 10 and 11 indicate that most activity is lost after three hours from the gamma iron/silica powder mixture and all is lost from the Mars analog B2 soil.

This question has been further examined using soil samples on which hydrogen peroxide has been shown (Figure 9) to be differentially stable for three hours at room temperature. In these experiments, hydrogen peroxide was added to the various samples and incubated for three hours at $<-70^{\circ}C$ under a nitrogen atmosphere at 760 mm Hg. The temperature was then raised to $10^{\circ}C$ and, after 48 hours at this temperature, nutrient was added to monitor the activity of any remaining hydrogen peroxide. The results were compared to control samples in which hydrogen peroxide was added to the sample at $23^{\circ}C$ followed by nutrient added either immediately or after 48 hours incubation at $23^{\circ}C$.

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The results of experiments conducted with several iron oxide samples are shown in Table III. For reactions conducted at room temperature, pre-incubation for 48 hours significantly reduces hydrogen peroxide activity with LR nutrient. All activity is destroyed by incubation with alkaline pH samples whereas, in agreement with previous results (Figure 8), some activity remains with the low pH samples. Pre-incubation for three hours at $<-70^{\circ}C$ followed by 48 hours at $10^{\circ}C$ preserves up to 50% activity with low pH (<3.8) samples but does not prevent complete destruction of hydrogen peroxide by high pH samples.

The mixture of 15% gamma iron and 85% silica sand was also tested in these experiments. This sample is of particular interest because TSM results (Figure 2) suggest that hydrogen peroxide may form a complex with this mixture at $<-70^{\circ}C$ that stabilizes it to volatilization at $50^{\circ}C$. The results are also shown in Table III. As shown, hydrogen peroxide on this sample at $23^{\circ}C$ has lower reactivity with LR nutrient than observed when placed on the other iron samples, probably because of the lower amount of stimulatory iron present in the mixture. However, hydrogen peroxide on this sample is considerably more stable than on the pure iron samples; 45% activity remains after 48 hours storage at room temperature and 70% remains after three hours at $<-70^{\circ}C$ followed by 48 hours at $10^{\circ}C$. These results provide further support that hydrogen peroxide could account

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TABLE III

Stability of Hydrogen Peroxide on Various Iron Samples
After Various Pre-Incubation Temperature Regimes

Iron Sample	pH	Soil Pre-Incubation Temperature	H_2O_2 Pre-Incubation Temperature	CPM Evolved from LR Nutrient in 25 Hours After 0 or 48 Hours H_2O_2 Pre-Incubation 0	48	% H_2O_2 Remaining Inhibition After 48 Hours
γFe_2O_3-MO2230	2.5	23°	23	80,000	9,000	11
		-70°, 10°	10	-	27,500	33
γFe_2O_3-MO4228	2.5	23°	23	138,000	2,000	1
		-70°, 10°	10	-	23,500	17
γFe_2O_3-COBALOY*	3.0	23°	23	98,000	1,800	2
		-70°, 10°	10	-	43,000	44
$\alpha FeO\cdot OH$-Goethite	3.8	23°	23	72,000	18,000	25
		-70°, 10°	10	-	36,000	50
$\gamma FeO\cdot OH$-Lepidociocite	8.0	23°	23	56,000	<1,000	0
		-70°, 10°	10	-	<1,000	0
γFe_2O_3-MAPICO	8.7	23°	23	<1,000	<1,000	0
		-70°, 10°	10	-	<1,000	0
γFe_2O_3/Silica Sand (15/85)	3.2	23°	23	40,000	18,000	45
		-70°, 10°	10	-	28,000	70

Aliquots (0.5cc) of the indicated samples were placed in sterile glass screw cap vials and the vials placed in a glove box under nitrogen at 760 mm Hg. After temperature equilibration at either room temperature (app. 23°C) or at <-70°C for 15 minutes, 0.22 ml of a 1.5 x 10⁻¹M hydrogen peroxide solution (pH 6.5) was added. LR nutrient (0.11 ml) was then added either immediately or after approximately 48 hours pre-incubation at either room temperature or reduced temperature. The reduced temperature pre-incubation regime consisted of three hours at <-70°C, 48 hours at 10°C, and 10 minutes at room temperature prior to nutrient addition. Radioactivity evolved following the nutrient addition war monitored by the getter technique. Percent hydrogen peroxide remaining after the pre-incubation was calculated by comparing radioactivity evolved from pre-incubated samples to that evolved from samples without pre-incubation and at room temperature.

* This sample was used for experiments reported in Figures 2,4,5,6 and 9 and for the mixture examined in Tables 1 and 2.

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for the active agent in the flight experiments, provided the
pH and composition of the Mars surface material were highly
acidic to stabilize hydrogen peroxide during the $10^{O}C$ storage.
However, because of the alkaline pH of the Mars sample suggested
by other experimenters (11,15), the inference must be interpreted
with caution.

In a final attempt to ascertain hydrogen peroxide
stability under conditions simulating those prevailing during
the Viking mission, an additional experiment was conducted in
which hydrogen peroxide was added onto either the B2 Mars analog
soil or the gamma Fe_2O_3/silica sand mixture (15/85%) contained
in 0.5 cc portions in liquid scintillation vials. Both soils
were pre-equilibrated on dry ice at $<-70^{C}C$ under 760 mm nitrogen
at the time of addition such that the hydrogen peroxide solution
froze instantaneously upon addition. (Sufficient hydrogen
peroxide was added such that the final concentration would be
$10^{-1}M$ following the later addition of LR nutrient.) Immediately
after adding the hydrogen peroxide, the vials and dry ice were
placed in a plastic glove box under nitrogen and transferred
to a walk-in freezer at $-30^{O}C$. All vials were maintained for 19
days in the presence and absence of ultraviolet irradiation (see
next section for discussions of UV exposure and effects) with
the temperature of the vials gradually rising from $-70^{O}C$ to
$-30^{O}C$ as the dry ice evaporated. At the end of the 19 day incuba-
tion, vials containing each soil (with and without UV) were

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separated into four groups to receive one of the following

treatments prior to addition of LR nutrient:

- Warm for 10 minutes at 10°C, then for 10 minutes at room temperature.

- Warm for 10 minutes at 10°C, then heat 3 hours at 50°C, then cool for 10 minutes at room temperature (simulates VL2-4).

- Warm at 10°C for 24 hours, then for 10 minutes at room temperature (simulates all active runs although on flight the storage time was about 48 hours).

- Warm for 36 days at 10°C, then for 10 minutes at room temperature (simulates long term storage in VL1-4 and VL2-5).

As a control for this experiment, an additional set of vials

was conducted in which hydrogen peroxide was added to soil at

room temperature and LR nutrient added immediately. This control

was conducted just prior to the 19 day storage using the same

hydrogen peroxide solution used in the storage portion of the

experiment. The entire experiment was conducted by the getter

technique with all getter pads counted in a Nuclear Chicago

gas flow counter at approximately 10% counting efficiency.

The results of this experiment are shown in Table IV.

For the gamma Fe_2O_3/silica sand mixture in the absence of UV,

approximately 20 percent activity remains after 19 days of cold

incubation followed by 20 minutes of warming prior to addition of

nutrient. However, only 12 percent remains if this warming period

at 10°C is extended to 24 hours. Thus, in order for sufficient

hydrogen peroxide to be present after 48 hours of warming at 10°C

to give a response of flight magnitude, its initial concentration

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Table IV

Effect of Low Temperature +UV Radiation on Subsequent Stability
of Hydrogen Peroxide on Mars Analog Soils to Treatment at 50°C and 10°C

Soil	Pre-Incubation Treatment of H_2O_2 on Soil	Subsequent Treatment	~CPM Evolved[a] 20 Hours After Add LR Nutrient	~% Activity Remaining
γFe$_2$O$_3$/Silica Sand (15/85%)	NONE	NONE	80,000	100
	19 days at -30°C, no UV	20 min at 10°C	16,000	20
		3 hours at 50°C	4,000	5
		24 hours at 10°C	9,500	12
		36 days at 10°C	1,000	1
	19 days at -30°C, +UV	20 min at 10°C	4,000	5
		3 hours at 50°C	5,000	6
		24 hours at 10°C	5,000	6
		26 days at 10°C	1,000	1
Mars Analog B2	NONE	NONE	16,000	100
	19 days at -30°C, no UV	20 min at 10°C	1,000	6
		3 hours at 50°C	500	3
		24 hours at 10°C	200	1
		36 days at 10°C	0	0
	19 days at -30°C, +UV	20 min at 10°C	1,000	6
		3 hours at 50°C	500	3
		24 hours at 10°C	200	1
		36 hours at 10°C	0	0

[a] Data corrected for CPM evolved from nutrient alone. Counting efficiency =10% or approximately threefold higher than on flight.

Aliquots (0.5cc) of the indicated samples were placed in screw cap vials and the vials placed on dry ice in a glove box under nitrogen at 760 mm Hg. After temperature equilibration for 15 minutes, 0.22 ml of a 1.5 x 10^{-2} M hydrogen peroxide solution (pH 6.5) was added. The vials were capped, moved into a plastic portable glove box under nitrogen at 760 mm Hg, and moved into a cold room at -30°C for 19 days. Vials were stored either capped or uncapped and exposed to an ultraviolet source at 254 mµ placed 4 inches above the soil surface to give a calculated exposure of 1620 µW/cm². After the 19 day incubation, vials of each type (i.e., each soil with and without UV) were divided into four groups which were then stored at 10°C for 10 minutes, 24 hours, or 36 days or heat treated for 3 hours at 50°C. After each of these treatments, vials stored at room temperature under nitrogen for 10 minutes prior to the addition of 0.11 ml LR nutrient. Radioactivity evolved following the nutrient addition was monitored by the getter technique. Percent hydrogen peroxide remaining after the various treatments was calculated by comparing to radioactivity evolved from samples in which the LR nutrient was added to vials at room temperature immediately after the hydrogen peroxide addition to the soil. All reactions were conducted in duplicate.

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would have had to be considerably greater than 10^{-1}M. (Note

that counting efficiency in the flight instruments was 3%;

since active flight responses ranged from 10,000 to 16,000 cpm,

these numbers must be multiplied by about 3 to relate them to

the data presented in Table IV). The remaining data with this

unexposed soil is in agreement with flight data. Thus, about

half of the activity seen after the 24 hour storage is lost by

three hours heat treatment at 50°C whereas almost all is lost

by long term storage (i.e., 36 days) at 10°C. (See next section

for discussion of UV effects.)

The results with the B2 Mars analog soil shown in

Table IV are essentially the same as those presented in Figure 11.

Thus, hydrogen peroxide is not stable when placed on this soil.

Even prior to the freezing regime, hydrogen peroxide reactivity

with the LR nutrient on this soil is considerably less than on

the gamma Fe_2O_3/silica sand mixture (see Table IV) despite the

fact that both soil samples contain approximately 15% gamma Fe_2O_3.

In summary of the Table IV results, it is apparent

that the extent of hydrogen peroxide stability on a soil sample

is highly dependent upon the composition and pH of that soil

sample. The results with the gamma Fe_2O_3/silica sand sample

suggest that, if a mechanism exists for formation and accumulation

of hydrogen peroxide on the surface of Mars, it may have been

possible for hydrogen peroxide to be present in the LR experiment.

However, in order to obtain a response with the LR nutrient of

flight magnitude, extremely high concentrations (i.e., $>10^{-1}$M

and approaching 1.0M) must have been present on the Mars surface

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if the gamma Fe_2O_3/silica sand mixture is a reasonable

representation of the Mars surface material. Alternatively,

either the Mars soil composition imparts a greater stability

to hydrogen peroxide or a metal catalyst is present which is more

effective than gamma Fe_2O_3. It should be noted, however, that

because the Mars analog B2 soil was prepared by the Viking

Inorganic Analysis Team to match the Mars Spectra (11), the

results obtained with this soil cast serious doubt on the ability

of hydrogen peroxide to survive on the surface of Mars. Finally,

perhaps the most difficult obstacle to be overcome is the

provision of some mechanism to protect the hydrogen peroxide

from destruction by UV - even when the type of otherwise

stabilizing complex formed in the laboratory is present.

D. Effect of Ultraviolet Radiation on Hydrogen Peroxide
    Stability

Hydrogen peroxide is highly susceptible to photolysis

by ultraviolet light which, on Mars, penetrates to the surface

virtually unattenuated by an ozone layer. In fact, it has been

reported that the rate of destruction on Mars favors the rate

of formation by a factor exceeding 10^7 (16,17).

The possibility that freezing or complexing could

protect hydrogen peroxide to ultraviolet irradiation was explored

this year. In a preliminary experiment, hydrogen peroxide placed

on either the gamma Fe_2O_3/silica powder mixture or on the Mars

analog B2 soil at $<-70^\circ C$ was exposed to ultraviolet radiation

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during subsequent maintenance at $<-70^\circ C$. This was accomplished

by removing the cap from the reaction vial containing the soil

plus hydrogen peroxide and placing a 254 mµ source approximately

4 inches above the soil surface to give a calculated dosage

to the soil surface of 1620 µW/cm^2. This exposure is approximately

one order of magnitude greater than that estimated (18,19) for

the Mars surface. Total incubation time at $<-70^\circ C$ was six

hours with UV present during the last three hours. As shown in

Figure 10, with the gamma Fe_2O_3/silica powder mixture, this

treatment enhanced subsequent reactivity with the LR nutrient.

With the B2 soil (Figure 11), a small stimulation was also

obtained; however, this stimulation was not observed when the

ultraviolet exposure was reduced from three hours to 20 minutes

(i.e., total pre-incubation at $<-70^\circ C$ was three hours and 20 minutes).

Because the short UV exposure time did not produce a

deleterious effect on hydrogen peroxide and because this result

is in contrast to the known (8,9) sensitivity of hydrogen

peroxide to UV photolysis, a further experiment was conducted

in which exposure times were extended to 19 days (Table IV). As

in the experiments cited above (Figures 10,11), the UV source

was a 254 mµ lamp placed approximately 4 inches above the soil

surface to give a calculated surface exposure of 1620 µW/cm^2.

Exposure was initiated at $<-70^\circ C$ under nitrogen although temperatures

gradually rose during the 19 day incubation to $-30^\circ C$ (see

previous section for experimental details). Soil samples selected

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were the gamma Fe_2O_3/silica sand mixture (15/85%) on which

hydrogen peroxide is known (Figure 6) to be more stable than

on the gamma Fe_2O_3/silica powder mixture, and the Mars analog

B2 soil. Following the 19 day radiation exposure, samples were

warmed and assayed with LR nutrient after storage for 10 minutes,

24 hours, or 36 days at $10^\circ C$ or after heat treatment for 3 hours

at $50^\circ C$. As shown in Table IV, in the two cases where any

activity remained, namely for storage of 10 minutes or 24 hours

at $10^\circ C$ on the gamma Fe_2O_3/silica sand mixture, the ultraviolet

exposure diminished hydrogen peroxide activity with LR nutrient

more than in samples incubated 19 days in the absence of ultra-

violet radiation. In all other cases, insufficient activity

remained even in the absence of ultraviolet exposure to

ascertain any further deleterious effect of UV. However, because

some activity remained in the UV exposed sample that simulated

the flight regime for an active response, the results do not

preclude the possibility that some hydrogen peroxide could have

been present with the Mars sample in the Viking LR experiment.

In this case, however, even higher concentrations would have had

to have been on the surface than indicated in the previous section.

Thus, the results seriously challenge the possibility that the

Mars sample composition could stabilize sufficient quantities

of hydrogen peroxide on the Mars surface to account for the LR

active responses.

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VI. Hydrogen Peroxide Formation on Mars Analog Soil

A necessary prerequisite to considering that hydrogen peroxide might be the active agent in the LR experiment is evidence for hydrogen peroxide formation under Mars environmental conditions. Mechanisms postulated for its formation include production in the Mars atmosphere coupled with freezing out on the planet surface [16,17] and ultraviolet induced formation by a reaction between water molecules and the Mars surface material [20].

Experiments reported earlier [4], in which Mars analog soil B2 was exposed to ultraviolet radiation for 712 hours under 5 torr carbon dioxide in the presence and absence of water vapor, failed to produce an agent that would react with the LR nutrient in the TSM. However, because these experiments could have been water limited, we have conducted additional getter-type experiments in a further attempt to generate hydrogen peroxide from water in contact with Mars analog soil. Variable amounts of water ($0,50,100,200 \mu l$) were added to the gamma Fe_2O_3/silica sand mixture at $<70^{\circ}C$ and incubated for three hours at that temperature. Duplicate samples were incubated in the presence and absence of ultraviolet radiation from a 254 mμ source at an estimated exposure of 1620 $\mu W/cm^2$ (approximately two orders of magnitude greater than on Mars). In no case was any evidence obtained for formation of an active agent.

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A similar experiment conducted in the TSM in which
100 μl water was added to this soil at -30°C under 5 torr carbon
dioxide in the presence and absence of water vapor failed to
produce an agent that would react with the LR nutrient.

In additional experiments conducted this quarter in a
further attempt to generate hydrogen peroxide from water in contact
with Mars analog soil, variable amounts of water (0,50,100,200 μl)
were added to the gamma iron/silica sand mixture at $<-70^{\circ}$C and
incubated for three hours at that temperature. Duplicate samples
were incubated in the presence and absence of ultraviolet
radiation from a 254 mμ source at an estimated exposure of
1620 μW/cm^2 (approximately two orders of magnitude greater than
on Mars). The temperature was then raised to 10°C and LR
nutrient added. In no case was any evidence obtained for formation
of an active agent.

A similar experiment conducted in the TSM in which
100 μl water was added to this soil at -30°C under 5 torr carbon
dioxide in the absence of UV also failed to provide evidence
for hydrogen peroxide formation.

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VII. Status of Biological and Chemical Interpretations of
      LR Flight Data

Several different kinetic components have been discerned

(4) in the active LR response obtained from the Mars surface

samples (1-3). These are reviewed diagramatically in Figure 12.

Following the initial nutrient injection, one and possibly two

first order reactions (#2a and #2b) occur concurrent with nutrient

outgassing (Reaction #1) and with a slow linear gas evolution

(Reaction #3). The contribution of Reaction #1 to the final

plateau range of 10,000 to 16,000 cpm appears to be less than

500 cpm, typical of results from nutrient alone experiments

routinely conducted in the LR TSM. The agent(s) responsible for

Reaction(s) #2 is thermally labile whereas that responsible for

Reaction #3 is thermally stable. Following the second nutrient

injection, an initial spike (Reaction #4) is followed by gas

absorption (Reaction #5) and a subsequent slow linear gas

evolution (Reaction #6). These latter three reactions are also

thermally stable. The similarity of Reactions #3 and #6 suggests

that a single agent is responsible for both reactions.

Our laboratory program has sought an explanation for

each of these reactions and all except Reaction #2 can now be

accounted for by physico-chemical phenomena (4,12). On the

other hand, the thermal lability of the Mars agent responsible

for Reaction #2 remains consistent with a biological explanation.

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Figure 12

Components of Labeled Release Reaction on Mars
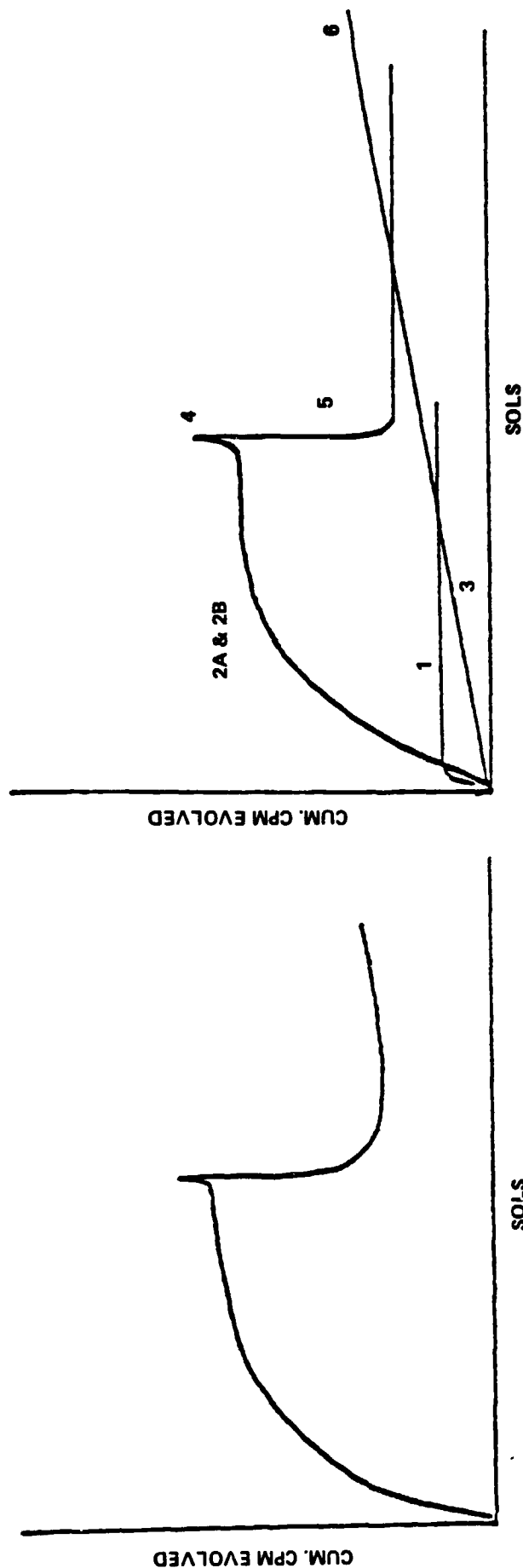
B) Component Reactions

1 – Nutrient Outgassing
2 – Thermally Sensitive First Order Reactions(s)
3 – Thermally Stable Linear Reaction
4 – Nutrient Outgassing and/or Pressure Spike
5 – Gas Adsorption
6 – Thermally Stable Linear Reaction

A) Overall LR Response

Because the biological interpretation is not subject to
direct experimentation but can only gain strength through
elimination of candidate nonbiological explanations, we have
undertaken an intensive systematic examination of the plausibility
of the various chemical alternatives. Our previous efforts (4)
have shown that ultraviolet irradiation, gamma Fe_2O_3, and
metalloperoxides and superoxides can be eliminated as candidate
active agents for Reaction #2. Under select conditions, however,
hydrogen peroxide can fulfill several, but not all, of the
necessary criteria for the Mars active agent.

Major problems remain with the hypothesis that hydrogen
peroxide is the active agent in the Mars surface material. Most
significant among these is demonstration that hydrogen peroxide
can be formed and survive on the Mars surface in quantities
sufficient to produce an LR response comparable in magnitude to
the flight response. Our experiments to date have not demonstrated
hydrogen peroxide formation under Mars-like conditions. If the
gamma Fe_2O_3/silica sand mixture can be considered a reasonable
Mars model soil, then our experiments permit a rough calculation
of the hydrogen peroxide concentration in the Mars soil that
would produce a LR positive response:

> As seen in Table IV, when 0.5cc of gamma
> Fe_2O_3/silica sand, which had been pretreated
> by holding at $-30^{\circ}C$ under UV flux equivalent
> to 190 days on Mars, and which had then been

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held at $10^{\circ}C$ for 24 hours, was injected with 0.22 ml of $1.5 \times 10^{-1}M$ hydrogen peroxide, a response totalling 5,000 cpm was produced. Adjusting for the higher counting efficiency of the getter technique, this equates to a response in the viking instrument of approximately 1,600 cpm, about cne-tenth that obtained on Mars. This indicates that, to produce a Mars-type response, a hydrogen peroxide concentration of 1.5M would be necessary.

A 1.5M solution of H_2O_2 contains

$1.5 \times 34 = 51$ g/l $= 51$ mg/ml.

0.22 ml $\times 51$ mg/ml $= 11.22$ mg H_2O_2.

This amount in the 0.5cc sample would produce a Mars magnitude response.

Assuming a specific gravity of 1.0 for the soil, the concentration of H_2O_2 would be

$$\frac{11.22 \times 100}{500} = 2.24\%.$$

Thus, to have been responsible for the LR Mars response, hydrogen peroxide would have to constitute more than two percent of the Mars soil. However, since the LR nutrient may only partially wet the soil, at least initially, the hydrogen peroxide concentration in the soil may have to be higher than 2%. Because hydrogen peroxide on the surface is suceptible to destruction by ultraviolet radiation, and to Mars temperatures approaching $10^{\circ}C$,

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a mechanism must also exist for formation of surface hydrogen

peroxide at even higher concentrations and for frequent replenish-

ment in the course of a year.

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The only possible means of lowering the required concentration while still producing a response of flight magnitude would be to have a more effective metal catalyst in the Mars soil and/or to form a more stable complex in the Mars surface material. Even then, the problem of survival of hydrogen peroxide under the Martian UV flux remains.

Another major difficulty with the hydrogen peroxide interpretation is the number of constraints imposed on the composition of the Mars soil. Thus, although our laboratory experiments have demonstrated that complex formation with analog soil can stabilize hydrogen peroxide to volatilization at $50^{\circ}C$, can partially stabilize it to decomposition on Mars soils even in the presence of UV by storage at low temperatures (i.e., $-30^{\circ}C$ or less), and can stabilize it to the two sol storage at $10^{\circ}C$, these stabilizations are highly soil dependent. To meet all criteria, the soil must be highly acid and contain both silica and iron (gamma iron, according to our experiments). However, it should be noted that the Mars surface material is believed to be of neutral (11) or high pH (15) and that the Mars analog soil B2 that matches the Mars inorganic spectra does not convey these stability properties to hydrogen peroxide. Thus, while such complexing can exist under certain specific conditions, it remains to be demonstrated that it is in fact possible with the Mars surface material.

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There is yet another constraint to be imposed on any complex between hydrogen peroxide and the Mars surface material. During the mission, the Mars active agent had been dissipated before the second nutrient addition, presumably either because it was limiting or because the experimental conditions had destroyed any remaining active agent. In our TSM experiments, excess hydrogen peroxide was not destroyed by the assay conditions but was available for interaction with nutrient added five days after the first nutrient injection. This implies either that if hydrogen peroxide were the active agent on flight, it was either limiting or complexed with surface material in such a manner as to render it susceptible to destruction by assay conditions. However, to produce a response comparable in magnitude to the flight response, hydrogen peroxide in our experiments has been approximately three orders of magnitude in excess of the amount of carbon oxidized on flight. Thus, in order for hydrogen peroxide to be limiting and account for the flight data, concentrations must be significantly lowered and a metal catalyst must strongly stimulate the reaction to replicate the magnitude of the flight response. Our experiments with pure gamma iron have not demonstrated stimulatory effects of this magnitude.

Thus, while the hydrogen peroxide theory remains an attractive chemical explanation of the LR flight data, several problems remain with this interpretation that probably cannot be resolved short of another mission. Most serious is whether

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hydrogen peroxide can form and persist in sufficient amounts

on the Mars surface material under Mars environmental conditions.

On the other hand, a biological explanation can also

account for the observed flight data and we have previously

discussed (4) various terrestrial life forms that can serve

as models for putative Mars organisms. These include cryptobiotic

bacteria and invertebrates, lichens, and endolithic algae, fungi,

and bacteria that exist beneath rock surfaces in Antarctica's

dry valleys. Such models lend some plausibility to a biological

interpretation for the positive LR response found on Mars. In

this regard, our study of the Viking lander pictures reporting

(5) greenish patches on some rocks near Viking Lander 1 and some

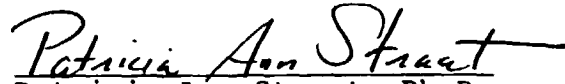changes in color pattern with time is of considerable interest.

One of the major obstacles for acceptance of a

biological interpretation of the Viking LR results has been the

failure of the Viking Organic Analysis experiment to detect

organic compounds in the Mars surface material (21,22). However,

the possibility exists that the sensitivity of the mass

spectroscopy experiment may not have been sufficiently low.

Thus, on theoretical grounds, we have estimated that a soil sample

must contain approximately 5×10^8 microorganisms per gram in

order to detect specific compounds or pyrolysis products.

This estimate is based on 1) a dry weight of approximately

5×10^{-4} g carbon/organism which would require 5×10^5 organisms

per gram soil to provide 1 ppb total carbon assuming 50% of cell

weight is carbon, 2) the assumption that two orders of magnitude

higher would be required to obtain 1 ppb of a specific compound,
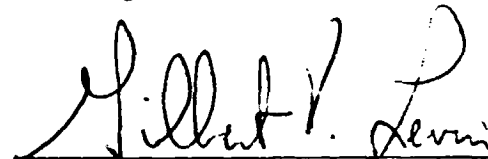
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and 2) detection limits in the Viking Organic Analysis experiment

of 5-10 ppb for benzene and toluene. This estimate can be

contrasted with the fact that positive LR responses have been

obtained from a terrestrial sample containing 10^2 microorganisms/

gram and that responses of flight magnitude are estimated to

require 10^4 cells/gram terrestrial soil. Thus, all aspects of

the Viking results are compatible with the biological hypothesis

while considerable problems remain to be solved to raise the

chemical hypothesis to that status. However, the paramount

importance of making a correct conclusion with respect to the

LR data requires further investigation, preferably by means of

a new mission to Mars.

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Respectfully submitted,

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